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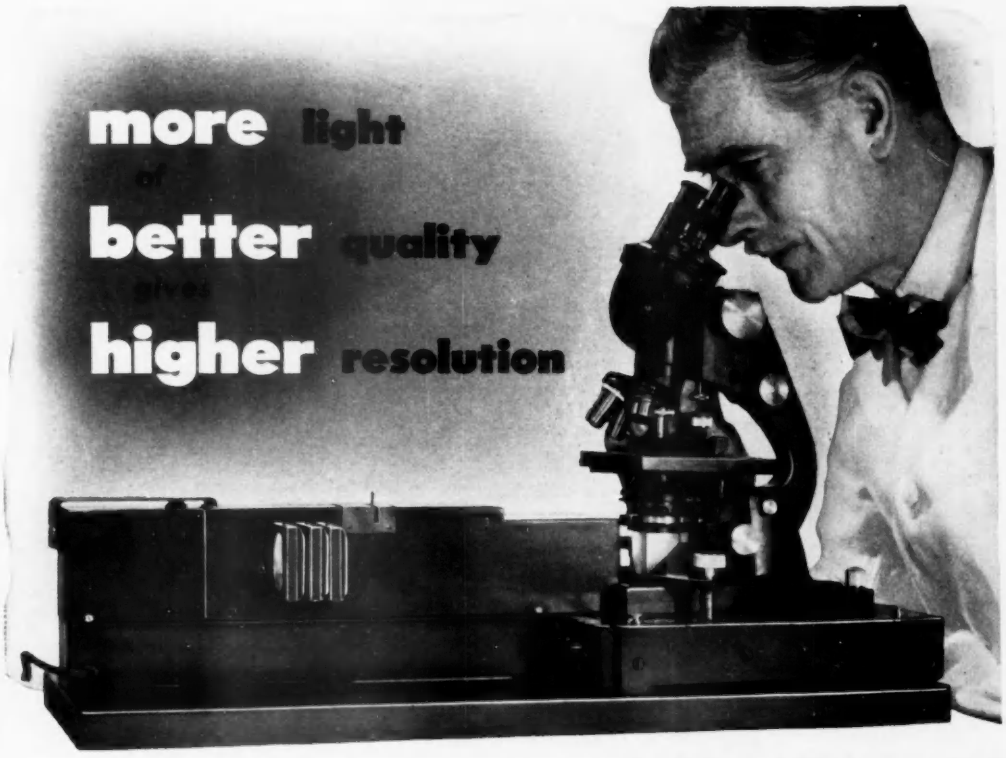

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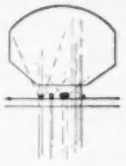

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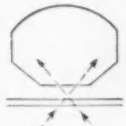

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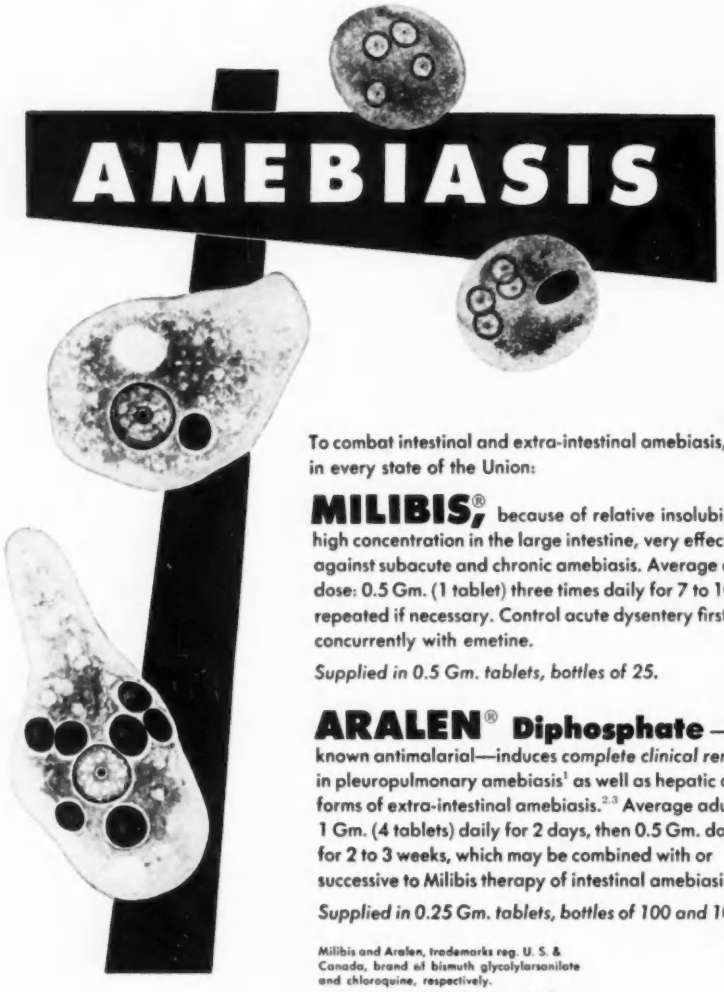

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2. Conan, N. J., Jr.: *Am. Jour. Med.*, 6:309, Mar., 1949.
3. Emmett, J.: *J.A.M.A.*, 141:22, Sept. 3, 1949.

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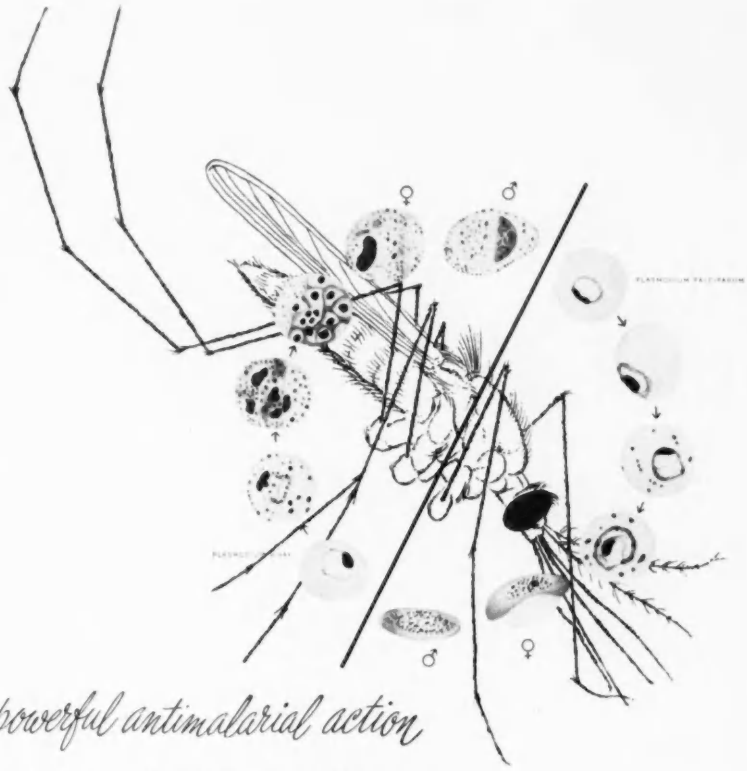

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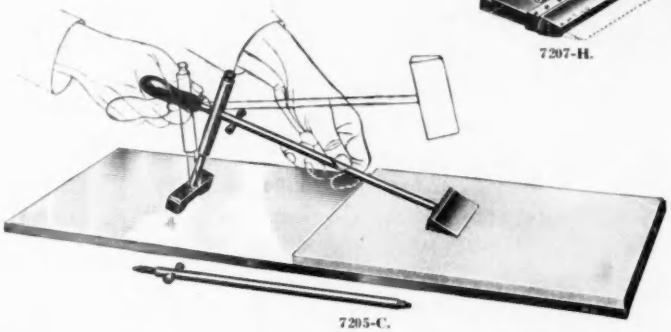

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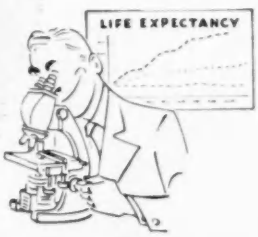

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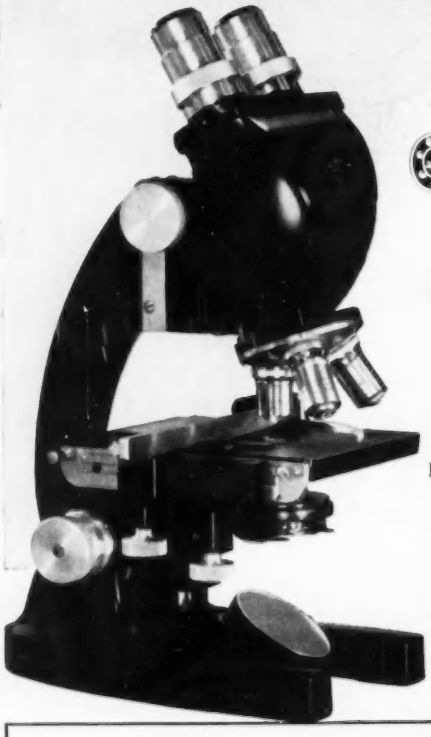

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COMPARISON OF THE EFFECTIVENESS OF THE EXAMINATION OF MULTIPLE STOOLS AND PROCTOSCOPIC MATERIAL FOR THE DETECTION OF AMEBIASIS

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The abundant literature on amebiasis which was reviewed by Dr. D'Antoni (1949) in his Presidential Address of 1948 indicates a widespread interest in this disease. A marked lack of agreement is evidenced, however, on certain aspects as fundamental as the prevalence of the infection.

The incidence of amebiasis found in a given group depends on several factors. Assuming adequate technical ability of the investigator these are: (1) the basic endemic incidence; (2) the selection of cases; (3) the technics employed.

BASIC STUDY

Parasitology at Charity Hospital is primarily a diagnostic service. Records kept according to specimens rather than patients examined seem best suited to our needs. The incidence of the various amebas encountered in the past two years is shown in Table I.

We believe that these data which show that *E. histolytica* is found in approximately 4 per cent of the specimens examined approach the correct figure of the endemic incidence of this area.

PRESENT STUDY

The Department of Pathology of Charity Hospital has cooperated with the Tulane University Department of Tropical Medicine and Public Health and the Louisiana State University Department of Medicine in two proctoscopic clinics. The purpose was three-fold: (1) to increase the efficiency of the services; (2) to determine the incidence of amebiasis in individuals who present gastrointestinal complaints; and (3) to compare the relative effectiveness of the examination of multiple stools and proctoscopic material for the detection of amebiasis. Only the last two lend themselves to analysis. Work in these clinics offered a satisfactory approach to the selection of cases. Sawitz and Faust (1942) showed that six consecutive fecal examinations using the direct method and Zn SO_4 concentration technics, supplemented by iron hematoxylin stains, reveal 95 per cent of known amebic cases. The work of Tobie *et al.* (1951) showed little advantage in the use of the combination of direct examination and the zinc sulfate concentration technic over the latter alone if the amebas encountered were primarily in the cystic stage. As our project was to deal exclusively with patients who complained of gastro-intestinal irregularity we adopted the plan of study shown in Table II.

Two hundred fifty-two patients were proctoscoped in the two clinics during

the period of November, 1948 through October, 1951. Sufficient data for inclusion in this series were obtained on only 200 of the cases (Table III).

TABLE I

Incidence of Amebas Found at Charity Hospital of New Orleans; Data Based on Specimens Examined

YEAR (JULY–JUNE)	1949–50	1950–51
Total No. Spec. Exam.	9014	7877
Negative	66.15%	74.36%
E. histolytica	4.01	4.14
E. coli	8.37	9.77
E. nana	7.00	7.8
I. bütschlii	.41	.42
D. fragilis	2.03	1.93

TABLE II

Plan of Study

Examination of
- Three fresh stool specimens
  - Direct—saline and iodine
  - $ZnSO_4$ concentration
- Proctoscopic material
  - Direct—saline and iodine
  - Iron hematoxylin stain
- Complement fixation

TABLE III

Examinations Made

Number of cases	200
Fresh stools	
Direct—3 or more	141
Direct—2	35
Direct—1	24
Proctoscopic material	
Direct	200
Iron hematoxylin	149
Complement fixation	45

RESULTS

The complement fixation tests which were performed by the Atlanta division of the Communicable Disease Center of the United States Public Health Service showed no correlation with the other findings and were therefore discontinued. This test is still in the experimental stage. The sensitivity has been decreased to avoid false positive reactions. It is now designed primarily to detect cases of extra-intestinal amebic activity and fails to disclose intestinal amebiasis (Donaldson,

1950). Hussy and Brown (1950) have reported the same type of standardization with like results.

TABLE IV

Final Diagnoses of Cases Studied

November 1948–October 1951

Total Cases	200	
Amebiasis	26	13.0%
Colitis (non-amebic)	21	10.5
Carcinoma	13	6.5
Ulcers (Gastric, etc.)	9	4.5
Diarrheal diseases (non-amebic)	68	34.0
No diagnosis made	20	10.0
No disease found	43	21.5

TABLE V

The Relative Effectiveness of the Examination of Multiple Stools and Proctoscopic Material for the Detection of Amebas

Total number examined	200	
E. histolytica	26	13.0%
Proctoscopic Positive, Stools Positive	18	9.0
Proctoscopic Positive, Stools Negative	3	1.5
Proctoscopic Negative, Stools Positive	5	2.5
Stools Positive (18 + 5)	23	11.5
Proctoscopic Positive (18 + 3)	21	10.5

TABLE VI

The Relative Effectiveness of Direct Examination and Iron Hematoxylin Stains of Proctoscopic Material for Detection of Amebas

E. histolytica	21
Direct Positive Iron Hematoxylin Positive	17
Direct Positive Iron Hematoxylin Negative	3
Direct Negative Iron Hematoxylin Positive	1
Direct Positive (17 + 3)	20
Iron Hematoxylin Positive (17 + 1)	18

TABLE VII

OM *Other Protozoa Found in the 200 Cases Studied*

E. coli	11	5.5%
E. nana	14	7.0
I. bütschlii	2	1.0
D. fragilis	8	4.0
Giardia	5	2.5
Chilomastix	4	2.0
Trichomonas	8	4.0
Balantidium coli	1	.5

The final diagnoses on these 200 patients who had presented gastro-intestinal complaints are shown in Table IV.

The number of our cases found to harbor *Endamoeba histolytica* is lower than the 17 per cent reported positive in a somewhat comparable study made by Dr. H. H. Anderson and colleagues (1948) in Memphis. Their basic endemic incidence may have been higher and/or the selection of cases more specific.

The relative effectiveness of the examination of multiple stools and proctoscopic material as shown in this series is given in Table V.

The 21 positive proctoscopic specimens revealed amebas as shown in Table VI.

Other protozoa found during the examination are shown in Table VII.

SUMMARY AND CONCLUSIONS

1. Two hundred patients examined at the proctoscopic clinics of the medical departments of the Louisiana State University and Tulane Medical Schools, were studied for the presence of *Endamoeba histolytica* infection.

2. The increase of incidence of amebiasis (from 4 to 13 per cent) found in patients presented in the proctoscopic clinics over that reported from the parasitology laboratory of Charity Hospital can be accounted for in several ways.

a. Method of keeping records.

b. Selection of cases.

c. Multiple examinations of the cases studied.

3. Proctoscopic examinations frequently disclose findings of considerable importance and so have a distinct value in themselves. The above data, however, indicate that adequate examination of properly collected fresh stools is somewhat, though not significantly, more efficient than the examination of proctoscopic material for the diagnosis of intestinal amebiasis.

4. Direct examination of material removed during proctoscopy revealed a few more *E. histolytica* parasites than did examination of iron hematoxylin stains made on the same material.

5. No true superiority of method was demonstrated but it was again shown that the use of additional procedures and repeated examinations reveal an increased number of amebic infections.

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A CULTURE METHOD AS AN AID FOR ROUTINE DIAGNOSIS OF AMEBIC INFECTION IN CEYLON

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As intestinal amebiasis is a very common condition in Ceylon, every possible method of diagnosis should be explored. Direct microscopic examination of feces sometimes fails to detect cases because the number of amebae or cysts passed in the stools is too small. Concentration methods such as zinc sulfate flotation (Faust *et al.* 1939) can be applied only for the detection of cysts—this technique being unsuitable for specimens containing only trophozoites or precysts (Tsuchiya 1942). When specimens reach the laboratory several hours after collection, as is often the case in this country, the amebae tend to lose their motility and to round up. At this stage the average laboratory worker experiences difficulty in their identification without the aid of fixing and staining which is laborious and time-consuming (Velat *et al.* 1950). These difficulties could be overcome by a culture method which has two main advantages, viz. a) the presence of amebae in large numbers makes their detection surer and easier by the average worker; b) their motility in culture facilitates their identification.

Culture methods for the diagnosis of amebic infection are not popular among workers in this country. Doubtful opinions regarding their suitability have also been expressed by some workers including Faust (1941) and Paulson (1941). It was therefore thought advisable to select at first a medium that would yield the largest number of positive results. The general plan adopted was (1) to evaluate a number of media regarding their suitability for excystation of cysts and their ability to promote the growth of trophozoites from fecal specimens, (2) to assess the ability of antibiotics in cultures to bring about some degree of bacteriostasis, thereby promoting the growth of amebae, and (3) by using the results so obtained to select or devise the most suitable medium for the diagnosis of intestinal amebiasis.

Only positive specimens were cultured. These were of three varieties: a) containing only cysts, b) only trophozoites, and c) trophozoites and cysts. In about 80% of cases the specimens contained no pus, blood or mucus. Cultures were made within six hours of the collection of specimens and examined for four days by aspirating only one drop from each culture daily, irrespective of whether the result was negative or positive.

The following media were evaluated:—

1. L.E.S. (Inspissated egg slant covered by Locke's solution with 10 per cent inactivated human serum but without any glucose).
2. Inspissated sheep serum slant covered by buffered 0.75% saline with 10% inactivated human serum.
3. Inspissated human serum slant covered by Ringer's egg white solution.
4. St. John's (1932).
5. Balamuth's (1946) without liver concentrate.

The results of this evaluation are shown in Table 1. The difficulties experienced by us in the case of inspissated slants were as follows: The bacterial growth was very heavy and the amebae did not flourish. Heavy amorphous deposits also formed in the culture tubes and *Blastocystis* appeared in large numbers. The deposits, as well as the *Blastocystis* and the poor growth of amebae, acted dis-

TABLE 1

Evaluation of media

	BACTERIAL GROWTH AS INDICATED BY TURBIDITY	QUANTITY OF SEDIMENT	BLASTOCYSTIS WHICH CAUSED CONFUSION	AVERAGE NUMBER OF AMEBAE PER LOW POWER FIELD	NUMBER OF SPECIMENS EXAMINED	NUMBER OF SPECIMENS POSITIVE	EFFICIENCY OF MEDIUM	REMARKS
							%	
L. E. S. (inspissated egg slant covered by Locke's Sol. with inactivated 10 per cent human serum.	++++	++++	++++	P	10	4	40	Only few specimens were examined as the growth of amebae was poor and produced heavy sediment.
Inspissated sheep serum slant covered by buffered 0.75% saline with 10% inactivated human serum.	++++	++++	++++	1	12	6	50	Only few specimens were examined as the growth of amebae was poor and produced heavy sediment.
Inspissated human serum slant covered by Ringer's egg-white solution.	+++	+++	++++	1	7	4	57	Only few specimens were examined owing to limited supply of human serum.
St. John's.	+	+	+	3	41	30	73	
Balamuth's.....	+++	++	+	4	41	30	73	

P = Less than one ameba.
\+ = Scanty.
++++ = Heavy.

advantageously in the examination. On account of these drawbacks and the small number of positive results, the slants were given up in favor of the wholly liquid media, viz. St. John's and Balamuth's where the bacterial growth, the deposit and the *Blastocystis* were found to be less than in the slants. The percentage of positive results was also higher in the liquid media.

Although a higher percentage of positives was obtained in St. John's and Balamuth's media, the results were not uniform. Some specimens gave positive results

in one medium and negative results in the other. As it was thought that this discrepancy was caused by differences in bacterial growth in these two media they were mixed in two different proportions, as given below. These two new media so obtained were designated J.B.1 and J.B.2.

J.B.1.	St. John's medium in double strength	500 cc.
	Distilled water	470 cc.
	Balamuth's egg yolk infusion, undiluted and unbuffered	30 cc.
J.B.2.	St. John's medium in double strength	500 cc.
	Distilled water	440 cc.
	Balamuth's egg yolk infusion, undiluted and unbuffered	60 cc.

It was thought that one of these mixtures would provide a degree of bacterial growth optimum for the culture of amebae. The reaction of these two media, as well as that of St. John's which was not adjusted, was found to be between pH 8.0–8.2. Balamuth's medium was adjusted to pH 7.4.

J.B.1 gave a higher percentage of positives and was also found to promote the growth of amebae from specimens which gave little or no growth in St. John's and Balamuth's media.

An attempt was then made to ascertain the extent to which these four liquid media could be improved by the addition of antibiotics. Fifty additional specimens were examined for this purpose, each being cultured in eleven tubes, with 5 cc. of medium in each, as follows:-

4 tubes, one each of the four media without antibiotics (controls)

4 tubes, one each of the four media with 150 units of penicillin salt G (Glaxo) per cc. of medium.

3 tubes (omitting Balamuth's medium), with 1 mg. of streptomycin calcium chloride complex (Glaxo) per cc. of medium. (Balamuth's medium was not tested with streptomycin as the addition of this drug caused precipitation in the medium).

The addition of penicillin increased the percentage of positive results with all the media tested, from 72 per cent in each of St. John's and Balamuth's media to 92 per cent in the former and 84 per cent in the latter, and from 74 per cent and 68 per cent in J.B.1 and J.B.2 respectively to 96 per cent in each. Reduction of bacterial growth in penicillin-treated tubes was only slight as compared with the controls and the amebae survived for longer periods. Streptomycin was more effective than penicillin in reducing bacterial growth but it failed to produce a high percentage of positives, probably on account of the growth of fungi of the genus *Candida* which had taken place in streptomycin-treated tubes. It was also observed that *Pseudomonas aeruginosa* grew better in penicillin-treated media than in untreated tubes, more markedly in J.B.2 and Balamuth's media. This organism was detected by a color change in the medium to green on the 3rd or 4th day and the appearance of this organism synchronized with the reduction in the number of amebae. This, however, did not affect the diagnostic efficiency of the medium as the amebae grew for at least three days, by which time the diagnosis was made.

All the antibiotic-treated media gave a slow growth at the beginning. The

maximum growth, which was frequently heavy and which showed about 20 or more amebae per low-power field, was reached on the 2nd or 3rd day. From the 4th day onwards the amebae showed signs of losing motility and were rounding up.

Comment. The difficulties we experienced with inspissated slants referred to earlier have also been shared by Spingarn and Edelman (1948), who using a standard technique, obtained only 8 per cent positive results with specimens containing cysts. The modified medium J.B.1 employed by us yielded a higher percentage of positive results. This may be attributed to the optimum level of bacterial growth in J.B.1 caused by the addition of a small portion of egg yolk infusion to St. John's medium which was lacking in certain ingredients necessary for bacterial multiplication. A further modification of this medium which improved its diagnostic efficiency to a high level was effected by the addition of penicillin. It is possible that the beneficial effect of this antibiotic is due to the state of partial bacteriostasis which is brought about. It has been observed by

TABLE 2

Combined efficiency computed on comparative examination of 50 specimens

MEDIUM	WITHOUT PENICILLIN		WITH PENICILLIN	
	Efficiency of single medium	Combined efficiency	Efficiency of single medium	Combined efficiency
	%	%	%	%
St. John's	72	84	92	96
Balamuth's	72		84	
J. B. 1	74		96	
J. B. 2	68		96	

Chinn *et al.* (1942) and Balamuth and Howard (1946) that excessive growth of bacteria is responsible for the reduction in the numbers of amebae in the early phase of culture. Antibiotics, such as penicillin, prevent this initial excessive bacterial growth and by thus reducing the early mortality rate of the amebae in culture to a minimum, provide favorable opportunities for their multiplication.

Since an attempt was made to ascertain whether the use of a number of media increased the diagnostic efficiency, the combined efficiency was computed on the basis of comparative examination of 50 specimens. It was noted (Table 2) that the diagnostic efficiency was increased when two media were employed, but that the same efficiency could be obtained by culturing in J.B.1 or J.B.2 with penicillin.

Summary. A satisfactory method for the diagnosis of amebic infection by culture has been evolved by the modification of the standard liquid media (St. John's and Balamuth's). The study has been based on the examination of 50 fecal specimens.

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STUDIES OF THE PHYSICAL PROPERTIES OF A GROWTH FACTOR FOR *ENDAMOEBA HISTOLYTICA*[1]

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The detection of a growth principle for *E. histolytica* in autoclaved bacterial cells has recently been described (Karlsson *et al.*, in press). The present report deals with further studies of the sources and properties of this factor, hereafter referred to as the *E. histolytica* factor.

MATERIALS AND METHODS

The organisms and the media employed have been described previously (Karlsson *et al.*, in press). In the present experiments a purified culture of the anaerobic *Streptobacillus sp.* was employed for production of the growth factor. The previously employed culture has been found to be mixed (James, unpublished). The present pure culture does not produce gas, but apparently ferments glucose with abundant acid production.

The assay procedure has been modified by substituting *E. histolytica* with *Streptobacillus sp.* as the inoculum, for the ameba grown with organism *t*. The bacteria from the inoculum are inhibited with penicillin G and streptomycin, instead of the solution containing aureomycin and bacitracin. The modified procedure gives more satisfactory and more uniform results, as this strain of the ameba is larger and grows more abundantly. The present method is as follows: to Hansen's liver-proteose-peptone medium is added 0.1 ml. 50 per cent glucose, approximately 10 mgm. rice starch, 1 ml. 0.3 per cent sodium thioglycolate, 5000 units penicillin G, 10 mgm. streptomycin, and 1 ml. *E. histolytica* with *Streptobacillus sp.* inoculum. The test material is added to the appropriate tubes and an anaerobic seal applied. The results are recorded after 3–4 days incubation.

RESULTS

It was suspected from previous studies that the *E. histolytica* factor might be heat-labile, so a method of killing the pregrown bacteria, less destructive than autoclaving, was developed. Treatment of the 24 hour old culture of *Streptobacillus sp.* with 4 per cent formaldehyde for 2 hours caused complete sterilization as far as could be determined by subculturing. The cells were freed of formaldehyde by centrifugation and repeated washing with distilled water. This preparation had much more activity than the autoclaved cells reported previously.

Assay Curve: For studies of the effect of various conditions on the *E. histolytica* factor, the minimum amount of dead bacteria needed for maximum response

[1] Supported in part by the Abbott Laboratories, North Chicago, Illinois, and the National Institutes of Health, Bethesda, Maryland.

had to be known so that any destruction could be evaluated in a favorable range of activity. An assay curve was determined by varying the amount of cells added. The response was directly proportional until the maximum was reached, as is shown in Figure 1.

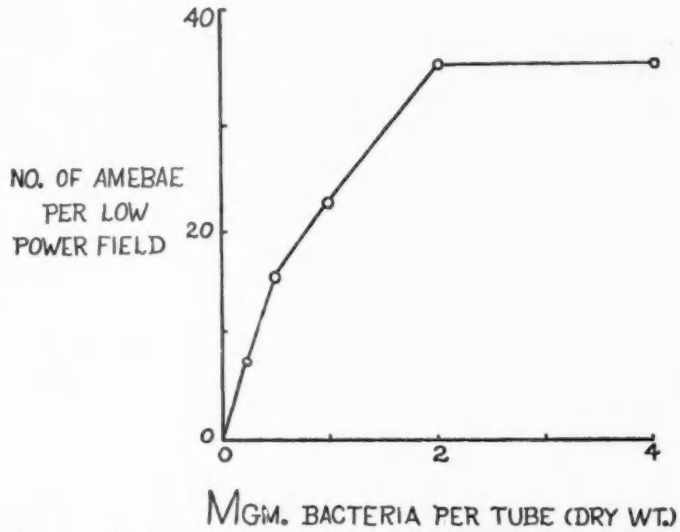

FIG. 1. Assay curve for effect of formaldehyde-killed bacteria on *E. histolytica*

TABLE I

Effect of the condition of Streptobacillus sp. *at the time of exposure to formaldehyde on the content of* E. histolytica *factor*

CONDITION OF BACTERIA	NO. OF AMEBAE PER LOW POWER FIELD	
	I	II
Active	35	45
Starved	0	0.2
Starved, then fresh medium	30	35
Starved, then glucose	30	30
Control without bacteria	0.8	0.9

Condition of Bacteria: Great variation had been encountered in the response to different samples of dead bacteria, although the treatment had been identical. This led to an investigation of the effect of the condition of the bacteria at the time of formaldehyde treatment. Table I shows that starvation by incubating the live bacteria for 1 hour at 37°C. in the absence of substrate before formaldehyde treatment resulted in complete loss of the activity. Restoration was accomplished by subsequent incubation in the presence of a nutrient substrate.

Storage: An experiment was done to determine the effect of storage in the refrigerator. Dead bacteria in an aqueous suspension retained all their activity during 10 days of storage.

Destruction by Heat: The minimum amount of bacteria for maximum response was suspended in 1 ml. of water in several test tubes. The tubes were autoclaved at 121°C. for varying lengths of time. The liver-proteose-peptone medium was added and the material tested as usual. Figure 2 shows that more than 90 per cent of the activity was destroyed during 20 minutes at 121°C. It should be pointed out that the precision indicated in Figure 2 is not representative of our methods, although it did happen in this experiment. Ordinarily the variation would be of a greater magnitude.

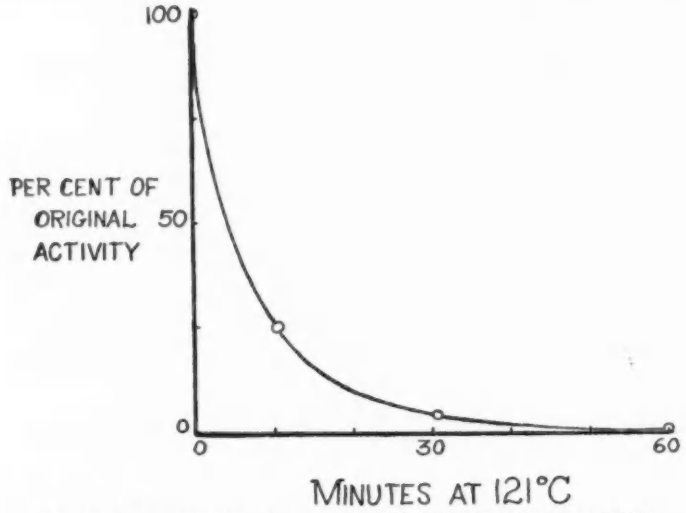

FIG. 2. Thermal destruction curve for *E. histolytica* factor at 121°C.

Effect of Acid and Alkali: Preliminary experiments had indicated that the factor was unstable in both 0.1N HCl and 0.1N NaOH at 100°C. When this experiment was repeated at room temperature for varying lengths of time, no destruction was caused by 0.1N HCl during 1 hour, whereas the factor was completely destroyed by exposure to 0.1N NaOH for 5 minutes.

Oxidation: Exposure of the dead bacteria to 3 per cent H_2O_2 for $\frac{1}{2}$ hour destroyed all the activity.

DISCUSSION

The data presented in this paper confirm the conclusions drawn in our previous publication. That the effect observed is caused by a growth factor and not by a physical change in the medium is indicated by the proportional response to the amount of bacterial cells which is seen in Figure 1.

The physical properties of the factor which have been studied indicate that it is fairly labile to heat, to alkali, and to oxidation. This limits the conditions under which the factor must be handled in future work. Since some of the properties coincide with those of coenzyme I, this compound was tested, but no activity was observed.

Although the factor seems to be closely bound to the bacterial cell, it is not likely to be proteinaceous in nature, judging from the heat-stability and from the resistance to formaldehyde. It is not extractable by fat solvents. The only clue as to its nature is the interesting observation that it disappears on starvation of the bacteria and reappears when metabolism is reestablished. This would suggest a labile metabolic intermediate.

Attempts are now under way to concentrate the factor from commercial yeast which has proved to be a more convenient and readily available source.

SUMMARY

The response of *E. histolytica* to a stimulating principle present in dead bacterial cells has been shown to be proportional to the amount added. The factor is rather labile to heat, to alkali, and to oxidation, but stable in presence of formaldehyde. There are some indications that it may be a labile metabolic intermediate.

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FUMAGILLIN IN AMEBIASIS[1]

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Fumagillin was isolated from *Aspergillus fumigatus* (NNRL 171) by Hanson and Eble (1949) . It was first described as an antiphage agent. While the chemical composition is not known, it does occur in crystals and has been reported to contain only carbon, hydrogen and oxygen. This antibiotic has shown slight antibacterial or antifungal activity. Its chief attribute as a direct acting amebicide was demonstrated by McCowen, Callender and Lawlis (1951). Against *Endamoeba histolytica in vitro*, fumagillin was reported active at one to four million in the absence of multiplying bacterial associates. In the presence of *Trypanosoma cruzi*, the amebicidal levels were found by Hrenoff and Nakamura (1951) to be from one to ten to 15 million dilution. The trypanosomes were not affected by these concentrations. Against experimentally induced rat and rabbit infections with *E. histolytica*, total doses of 11 mgm. per kilo orally in rabbits over two days proved to be amebicidal (McCowen *et al.*, 1951). On the basis of these initial observations it was believed desirable to study fumagillin in chronic amebiasis of macaques and man.

Fumagillin in Monkey Amebiasis

Following the method recently reported by Hrenoff and Nakamura (1951) and associates (Anderson *et al.*, 1950), the antibiotic was studied in 19 monkeys naturally infected with *E. histolytica*. The range of oral dosage, duration and effectiveness of therapy, during a three-month follow-up period are given in Table I.

Tolerance studies in infected animals were made to determine what toxic or other untoward effects might be anticipated in man. Within the range of effective dosage, one animal had continued retention of sulfobromophthalein (Bromsulphalein), and one other monkey had an abnormally high blood urea nitrogen level after therapy. Table II summarizes these results, which were otherwise essentially negative.

Against chronic *E. histolytica* infections, fumagillin cleared 12 of 19 animals which did not relapse during the three-month follow-up period. Other intestinal parasites were not consistently cleared, as indicated in Table III.

Comparative Studies in Man

On the basis of the preliminary observations in monkeys and the findings in other animals which were noted in the Upjohn Laboratories (Burbidge, pers.

[1] Received for publication November 17, 1951.

[2] With the technical assistance of Mrs. Betty Mythen, Miss Dorothy Swanman and Mr. Aram Demirjian.

TABLE I

*Range of oral dosage, duration and effectiveness of fumagillin in macaques**

3 given	50	mgm./Kg. daily for 5 times:	3/3	cleared
5 given	62.5	mgm./Kg. daily for 10 times:	3/5	cleared
6 given	100	mgm./Kg. daily for 5 times:	2/6	cleared
5 given	125	mgm./Kg. daily for 5 times:	4/5	cleared
			12/19	cleared

* Naturally infected with *E. histolytica* and results of therapy over three-month period of follow-up.

TABLE II

Studies of drug tolerance with amebicidal doses of fumagillin in macaques†*

Changes in:	
Weight‡	18 of 19 gained an average of 19%
Prothrombin time	None noted (in 5 animals tested)
Hemoglobin	14 of 19 increased an average of 0.8 Gm./% (S) 5 of 19 decreased an average of 1.05 Gm./%
Leukocytes (total)	8 of 19 increased an average of 2.43 M./cmm. 11 of 19 decreased§ an averageof 3.76 M./cmm.
Erythrocytes (total)	11 of 19 increased an average of 0.35 mill./cmm. 8 of 19 decreased an average of 0.42 mill./cmm.
Bromsulfalein	3 of 19 increased 10, 25 and 27%; all but 1 returned to normal after therapy completed.
Blood urea nitrogen	6 of 17 decreased an average of 4.8 mgm.%¶ 11 of 17 increased an average of 6.7 mgm.% 1 remained abnormally high (25.6 mgm.%) and 1 other which was high before therapy reduced to 22.3 mgm./% post therapy

* Observations in 19 animals over a 3-month follow-up period (1 died of miliary tuberculosis 4 weeks after therapy).

† No changes in electrocardiographic tracings were observed in any animal.

‡ Four of 5 given 62.5 mgm./Kg. daily for 10 days lost weight (3 to 17%) during therapy but regained it later.

§ No abnormally low total counts were recorded (↓ 10,000 WBC's/cmm.).

¶ Within the range of 10–20 mgm.%

TABLE III

*Effect of fumagillin against intestinal parasites in macaques**

12 of 19	cleared of *E. histolytica*
3 of 13	cleared of *E. coli*
1 of 12	cleared of *Iodamoeba buetschlii*
2 of 7	cleared of *E. nana*
1 of 1	cleared of *Trichomonas hominis*
0 of 1	cleared of *Chilomastix mesnili*

* Over three-month follow-up period.

comm.), cautious trial was begun in 20 chronically infected patients in Beirut.[1] Because some animals, notably dogs, had shown anorexia, vomiting and diarrhea, as well as uncomfirmed hypoprothrombinemic activity, at dose levels within the range we had used in monkeys, tolerance studies in man were included. Table IV summarizes our findings in man at single doses of 5 to 10 mgm. per day

TABLE IV

Laboratory studies on tolerance to fumagillin in man

	CASE NO.	CONTROL: BLOOD UREA N.	POST-THERAPY: BLOOD UREA N.	CONTROL: SERUM BILIRUBIN	POST-THERAPY: SERUM BILIRUBIN	CONTROL: PROTHROMBIN UNITS	POST-THERAPY: PROTHROMBIN UNITS*
		mgm/%	mgm/%				
Total dose = 50 mgm. in 12½ days	1	10.0	11.0	0.8	1.0	280	275
	2	10.0	11.0	0.6	1.0	314	297
	3	9.0	10.0	0.9	0.8	297	320
			11.0		0.7		277
	4	9.0	10.0	1.0	1.0	302	310
			10.0		0.8		264
	5	*P.S.P.*: 75% in 60 min.	*P.S.P.*: 70% in 60 min.	*Icterus Index:* 8.5	*Icterus Index:* 8.0	295	275
	6	*P.S.P.*: 75% in 60 min.	*P.S.P.*: 70% in 60 min.	*Icterus Index:* 8.8	*Icterus Index:* 9.0	320	334
	7	12.0	16.0	0.5	1.0	297	275
	8	15.0	15.0	1.0	1.0	335	320
	9	10.0	10.0	0.8	0.4	342	295
	10	12.0	15.0	0.9	1.0	325	255
Total dose = 100 mgm. in 12½ days	11	10.0	13.0	1.0	0.8	350	270
	12	15.0	15.0	1.0	1.0	285	285
	13	12.0	16.0	0.5	1.0	275	275
	14	*P.S.P.*: 75% in 60 min.	—	*Icterus Index:* 8.5	*Icterus Index:* 9.0	334	—
	15	*P.S.P.*: 75% in 60 min.	—	*Icterus Index:* 8.8	*Icterus Index:* 9.0	297	—
	16	15.0	14.0	1.0	0.9	277	275
	17	12.0	15.0	0.9	1.0	325	255
	18	10.0	15.0	1.0	0.8	264	265
	19	11.0	10.0	1.0	1.0	285	297
	20	11.0	15.0	1.0	1.0	297	335

* Method of Warner and Smith, Am. J. Phys., **114**: 667, 1936, as modified by Hurn, M., and Mann, F. D., Am. J. Clin. Path., **17**: 741, 1947.

for a total dosage of 50–100 mgm. orally over 12½ days. It is noted that there were no significant changes in blood urea nitrogen, serum bilirubin nor in prothrombin test levels. Since we were working with ambulatory students, staff and other campus personnel, larger doses were not used. No untoward effects of any kind were observed in this group.

Other laboratory findings which were noted in man are compared with similar

[1] Three uninfected volunteers in the laboratory took the doses indicated before the trial in patients.

observations in macaques as reported in Table V. In monkeys there was an increase in hemoglobin level more often than decrease (ratio of 3:1); a decrease in total leukocytes occurred about as often as an increase (11:8); and a reverse trend for erythrocytes.

In order to appraise fumagillin critically, a control group of ten untreated patients (cyst passers) was observed over three months or more, and two other groups, of 18 patients each, were given thiocarbarsone and terramycin respectively. These two agents had proved, in our hands, to be the most effective amebicides of those tested so far.

TABLE V

Comparative effects of fumagillin in monkeys and man infected with E. histolytica

	MONKEYS	MAN
Number treated	19	20
Number cleared	12	13
Dose/days	50–125 mgm./Kg./5–10	5–10 mgm. total/12½*
Effect on non-pathogens	insignificant	insignificant
Effect on prothrombin time	none	none
Effect on hemoglobin	3:1 increased:decreased	no significant change
Effect on leukocytes	11:8 decreased:increased	no significant change
Effect on erythrocytes	11:8 increased:decreased	no significant change
Bromsulfalein retention	3 increased; 1 permanently	none
Serum bilirubin	not tested	no significant change
Blood urea nitrogen	1 remained high	not abnormal
Electrocardiographic changes	none	not recorded
Other effects on fecal flora	no change in bacterial flora observed; nor in physical characteristics	no change noted in bacterial flora nor in character of stool

* Total dosage (highest) 100 mgm. given orally to 10 patients.

With terramycin, a preliminary report (Most *et al.*, 1951) had indicated that of six patients given this antibiotic alone, two relapsed after one to one and one half months. Total doses were from 9 and 13 Gm. respectively. The higher dose of 15 Gm. given over a period of five days was effective, according to an earlier report. In general, this antibiotic was well tolerated and the amebae disappeared within two to four days. Clinical improvement occurred at about the same time. There is no certainty, however, that terramycin is effective in hepatic amebiasis. In fact, our experience would indicate that this antibiotic should not be relied upon to control amebic hepatitis or when abscess of the liver is suspected.

The comparative clinical values of terramycin, fumagillin and thiocarbarsone (see Anderson *et al.*, 1949) in contrast to the untreated control group are given in Table VI, based on our human results in Lebanon.

The group of ten untreated control patients remained infected with *E. his-*

TABLE VI

Results of oral therapy of 66 patients with amebiasis in Beirut, Lebanon

DRUG	DOSE SCHEDULE	NO. OF PATIENTS	NO. CLEARED OF E. HISTOLYTICA	TIME OF RELAPSE AFTER THERAPY	CHARACTER OF RELAPSE	SUBSEQUENT THERAPY OF RELAPSED CASES	SIGNS AND SYMPTOMS OF DRUG TOXICITY OR INTOLERANCE	REMARKS
Control (no Rx)	—	10	none	—	—	—	—	Majority were symptom-free, cyst passers.
Thiocarbarsone	50 mgm. T.I.D. for 10 days; rest 10 days then course repeated.	18	16	2 months 4 months	*Clinical: bleeding* areas in sigmoid; *Parasitic:* + for *E. histolytica.*	Terramycin, 10 Gm. in 10 days; Fumagillin 100 mgm. in 12½ days.	Nausea in 4, headache in 3, abdominal pain in 2, diarrhea in 2, vertigo in 1, vomiting in 1.	One of two relapses believed to be due to re-infection (large race initially and small race noted 4 months after therapy).
Terramycin	250 mgm. T.I.D. or Q.I.D. for 10 days.	18	14	2½ months 2 months 2 months 1 month	*Parasitic:* + for *E. histolytica; Clinical:* hepatic symptoms & signs (enlarged & tender liver) in 3 patients.	Chloroquine, 6.3 Gm. in 21 days plus Fumagillin, 50 mgm. in 12½ days; *for 3* others, Chloroquine, 7.5 Gm. in 14 days; one also given thiocarbarsone, 1.5 Gm. in 10 days.	Nausea in 3, weakness in 2, anorexia in 1.	Patients with hepatic amebiasis not cleared after terramycin.
Fumagillin	5 mgm. alternate days, 3 x; then daily for 7 days.	10	4	1 to 3 weeks	*Parasitic:* + for *E. histolytica* in all cases.	*Fumagillin* at higher dosage; 100 mgm. in 12½ days.	Pains in back and abdomen in 1 (? drug)	Dose range below effective level; six not cleared of *E. histolytica.*
	5 mgm. daily for 5 days then B.I.D. for 7½ days.	10	9	1 week	*Parasitic:* + for *E. histolytica.*	—	Constipation and diarrhea in 1 (? drug)	Tuberculosis may account for intestinal complaints.

+ Based on 10+ stools examined over 3 to 6 months, except for fumagillin were 7.5+ examinations were made over 3 months.

tolytica throughout the control period (3 to 6 months). This is the first opportunity we have had to use such controls as a base-line for determination of drug effectiveness in other groups treated simultaneously. Placebos were given to the control group without change in clinical status; *E. histolytica* were demonstrated during the following-up period.

The patients treated with thiocarbarsone responded in a satisfactory manner, since 16 of 18 were cleared of the ameba. However, this group had the greatest number of untoward drug effects, but in only one case were these of sufficient severity to preclude continuation of therapy. It is possible that in one patient re-infection rather than relapse occurred. The large race of ameba observed before therapy was not seen four months after therapy was completed, but a small race of *E. histolytica* was demonstrated.

Terramycin was effective in clearing 14 of 18 patients of the pathogenic ameba or of the clinical symptoms. In one of the four relapsed cases, the ameba was not cleared, while in three others signs and symptoms of hepatic amebiasis persisted. In the latter patients, on recommendation of Dr. Neal Conan, chloroquine was successfully administered. Thus, it would appear that terramycin may be effective against the pathogenic ameba in the bowel, but cannot be relied upon for control of hepatic amebiasis, nor abscess of the liver. The abscessed liver responds usually to aspiration and specific "hepatic" amebicides, such as chloroquine or emetine.

Results with Fumagillin

Of the newer antibiotics, fumagillin appears to be most promising as a direct acting amebicide. Among the twenty patients, summarized in Table VI, none has shown any significant clinical effect of an untoward nature. The oral doses employed were very small, in comparison with dose levels of other amebicides (about $\frac{1}{6}$ the dose of emetine). Also, in comparison with doses employed in macaques, the total dosage for any patient was less than the single mgm. per kilo dose for experimental animals. The *in vitro* amebicidal level of fumagillin, reported by McCowen, *et al.*, was significantly greater than that of any other amebicidal agent. Since the action of this antibiotic against non-pathogenic intestinal protozoa is not significant, one should be certain to distinguish between *E. histolytica* and *E. coli* during follow-up examinations.

It is not possible to predict whether the extremely small doses employed are within the certain effective amebicidal range. However, the antibiotic appears to be tolerated, in the dose range employed, without harmful effects on the vital functions of man. Double and larger doses have been used in Egypt without harm (Killough, pers. comm.). Whether the drug is effective in the control of acute dysentery or against hepatic amebiasis is not possible to predict from our findings. In nine of the twenty patients, another amebicide had been employed prior to fumagillin therapy without success. Thus, it may well be that this antibiotic will find a place in human amebiasis therapy, particularly for the control of cases not responsive to currently available amebicides. Since it has not shown significant activity against associated bacteria, either *in vitro* or in monkeys or

man, its direct amebicidal effect makes it unique among the antibiotics now available for human use. However, it should be stressed again that its place in antiamebic therapy is yet to be established by subsequent studies. It would appear to offer greatest value in chronic, drug refractive, intestinal amebiasis.

SUMMARY

Fumagillin, orally, is active directly against *E. histolytica* in macaques and man. In man, at a total dosage of 100 mgm. over 12½ days, 9 of 10 cyst passers remained clear during 3 months; 50 mgm. total cleared only 4 of 10 patients. In monkeys, total dosage of 250–625 mgm./Kg. over 5 to 10 days was required to keep 12 of 19 animals cleared during 3 months.

No signs nor symptoms of intolerance were noted in man. In monkeys, one showed retention of sulfobromophthalein while another had increased blood urea nitrogen after therapy.

Other laboratory findings were not significant in either monkeys or man. Nonpathogenic intestinal parasites did not clear consistently in either mammalian species. There was close correlation between results in monkeys and man generally.

A comparative study with untreated controls and with terramycin and thiocarbarsone treated patients was made. Fumagillin was most potent and appeared to be most active in chronic, drug refractive, intestinal amebiasis.

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THE EFFECT OF TWO DIFFERENT DIETS ON EXPERIMENTAL AMEBIASIS IN THE GUINEA PIG AND IN THE RAT

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As a continuation of the previous work on diet (Taylor *et al.* 1950) as an influencing factor in experimentally induced infections of *Endamoeba histolytica* in the guinea pig, the effects of two additional diets have been studied in both the guinea pig and the rat.

A summary of most of the pertinent literature on the influence of diet on intestinal protozoan parasites is given in Table 1. It is obvious that much of the work was done before the era of synthetic or purified diets. There are, however, certain consistencies in the results: (a) diet influenced the numbers and/or symptoms produced by intestinal protozoa; (b) diets high in animal protein reduced the number of parasites and/or symptoms (the canned salmon diet in the dog is an exception); (c) the effects were seemingly consistent among parasite species and among the host species used.

The effect of diet on the amebic infection produced experimentally in guinea pigs and rats was important to us in that we needed a consistently high percentage of infections before undertaking a testing program to discover antiamebic compounds. One of the diets described here increased the percentage of induced infections in both the guinea pig and the rat, while the other gave a high incidence of amebic infection in the rat and the reverse effect in the guinea pig.

MATERIAL AND METHODS

Guinea pigs

Guinea pigs of both sexes, 10 to 21 days old, and weighing 85 to 250 grams have been used in these studies. They were housed one per cage to minimize the possibility of cross-infection. Food and water were supplied *ad libitum*. Weights were recorded the day that the animals arrived in the laboratory and every other day thereafter to the end of the experimental period, 30 days after the inoculation of amebae.

The guinea pigs were brought to the laboratory seven days prior to inoculation and placed in test groups of 10 animals each; the diets to be tested were fed from that day to the end of the experimental period.

The modified guinea pig (MGP) diet contained the following constituents: 50 per cent dry skim milk; 25.7 per cent sucrose; 17.8 per cent alfalfa leaf meal; 4 per cent salt mixture* (Hubbel, Mendel, and Wakeman); 2.5 per cent fat (Crisco). To each 1,000 grams of the diet the following vitamins were added: 10 mg. thiamine*, 14 mg. riboflavin*, 10 mg. pyridoxine*, 30 mg. calcium pantothenate*, 2 gm. inositol*, 100 mg. niacin*, 100 mg. *p*-amino benzoic acid*, 0.40 mg. biotin*, 3.0 mg. folic acid*, 300 mg. choline, 2 drops oleum percomorphum, 12 mg. caro-

* Quantities as recommended by Booth, Elvehjem and Hart (1949).

TABLE 1

Review of literature on the influence of diet on intestinal protozoa

HOST	INTESTINAL PROTOZOA	DIET	PARASITES	REFERENCE
Rat	*Giardia muris* *Hexamitus muris* *Trichomonas muris*	High Protein	Reduced	Hegner (1923)
Rat	Same as above	Carnivorous	Reduced	Hegner (1924)
Rat	*Councilmania muris* *C. decumani*	Skim milk	Cleared	Kessel (1924)
Rat	*Endamoeba coli*	Milk and Lacto-Kelpol	Cleared	Kessel (1929)
	Endamoeba coli	Regular ration and lactose	Cleared	Kessel (1929)
Rat	Amoebae	High Carbohydrate	Increased	Hegner and Eskridge (1937)
		High Protein (casein)	Reduced	
		High Protein (liver)	Reduced	
Rat	*Trichomonas muris*	High protein	Reduced	Ratcliffe (1928, 1929, 1930)
	Trichomonas hominis	High protein		
	Pentatrichomonas ardin delteili	High protein		
Monkey	*E. coli*	Raw milk	Cleared	Kessel and Huang (1929)
	E. histolytica	Raw milk	Reduced	
	Endolima	Raw milk	Reduced	
	Iodamoeba	Raw milk	Reduced	
Monkey	*E. histolytica*	Milk, lactose and Lacto-Kelpol	Cleared	Kessel (1929)
Monkey	*E. histolytica*	Autoclaved rice		McCarrison (1919)
Dog	*E. histolytica*	Raw liver and liver products	Controlled dysentery	Kagey and Faust (1930)
Dog	*E. histolytica*	Cod liver oil	Controlled	Faust (1932)
Dog	*E. histolytica*	Canned salmon	Increased	Faust *et al.* (1934)
Dog	*E. histolytica*	Canned salmon	Increased	Thompson and Lilligren (1949)
Rabbit	*E. histolytica*	Bread grain	Increased	Westphal (1941)
	E. histolytica	Bread grain	Increased	Tobie (1949)
Man (children)	*E. histolytica*	Raw milk	Reduced	Kessel and Huang (1926)
Man	*E. histolytica*	Milk, Lacto-Kelpol and lactose	Reduced	Kessel (1929)
Man	*E. histolytica*	Modified D'Antoni (18) High protein, low carbohydrate	Reduced	Felsenfeld and Comess (1947)

TABLE 1—*Continued*

HOST	INTESTINAL PROTOZOA	DIET	PARASITES	REFERENCE
Man	*E. histolytica*	High protein, low carbohydrate	Reduced	Shlaes and Felsenfeld (1949)
Man (Bantus)	*E. histolytica*	Maize	Increased dysentery	Elsdon-Dew (1949)
Man (Indians)	*E. histolytica*	Curry and rice	Rare liver abscess	Elsdon-Dew (1949)
Man	*E. histolytica*	Deficient diets	Increased	Sautet *et al.* (1948)
Guinea pig	*E. histolytica*	Crude diets deficient in ascorbic acid	Increased	Sadun *et al.* (1950)
Guinea pig	*E. histolytica*	Purified diet deficient in ascorbic acid	Increased	Sadun *et al.* (1951)

tene, and 0.20 mg. vitamin K. Twenty-five milligrams of ascorbic acid in sugar solution were given orally to each animal every other day. The rat breeder diet† consisted of the following: 33 per cent ground corn meal, entire kernel; 31 per cent ground whole wheat; 7 per cent linseed oil meal, expeller type; 2 per cent alfalfa meal; 21 per cent dried whole milk; 3 per cent dried hog liver; 2 per cent strain G yeast (Anheuser-Busch); 0.5 per cent sodium chloride; 0.5 per cent calcium carbonate. The supplement of ascorbic acid was also given.

Trophozoites of strain 200 (Tobie 1949; Taylor *et al.* 1950) of *E. histolytica* grown with an undetermined bacterial flora were employed throughout the investigation. The cultures were transferred every 48 hours in Locke-egg-slant-Locke-overlay medium plus rice powder. The inoculum was from 24-hour cultures. Following laparotomy 0.5 ml. of the inoculum was injected intracecally by a technique which has been described in detail previously (Taylor *et al.* 1950).

An autopsy was performed on each animal at death or at the end of the experimental period. The cecum and colon were examined for gross ulcerations and fresh preparations of the cecal content were examined microscopically for the presence of *E. histolytica*. The degree of infection produced in an animal was graded according to the following scale: no gross ulcers or amebae in cecum, 0; amebae (trophozoites) present in the cecal smear, 1; a few ulcers of the cecum and presence of amebae, 2; numerous ulcers over the cecum and presence of amebae, 3; numerous ulcers of the cecum and the colon and presence of amebae, 4. An average degree of infection (ADI) was obtained for each experimental group. Those animals that died within two days after inoculation were considered to have died as a result of the operation and hence were excluded from the calculations.

† We are indebted to Dr. Paul Thompson, Parke, Davis & Company for the formula of this diet.

Rats

Weanling rats of the Osborne-Mendell strain, weighing 30 to 104 grams, were placed on the test diets immediately after they arrived in the laboratory and were inoculated with trophozoites of *E. histolytica* two days later. They were housed five per cage and each test group consisted of 10 animals. The animals were weighed immediately prior to inoculation and again a week later at the time they were killed and autopsied.

The method of inoculation was similar to that of Jones (1946) except that mucin was not added to the inoculum and the inner muscular layers were not sutured; the outer body wall was closed with Michel clips. The amount of inoculum injected into each animal was 0.25 ml. The amebae were from the same source and treated in the same manner as those described in the section on the guinea pig.

The evaluation of the degree of infection is essentially that of Jones (1946). The ceca were examined for gross pathological changes and the cecal contents were examined for the presence and abundance of amebae. The degree of infection was estimated according to the following scheme: no gross pathological signs and no amebae, 0; no pathological signs, few amebae, 1; no pathological signs, many amebae, 2; thickening of and/or mucus on part of the cecal wall, amebae present, 3; thickening of and mucus on all the cecal wall, amebae present, 4; ulcers present, and amebae, 5. An average degree of infection was obtained for each group. Only those animals that survived the 7-day experimental period were included in the calculations.

EXPERIMENTAL

Guinea Pigs

In the first work reported (Taylor *et al.* 1950) the percentage of infections produced by *E. histolytica* in guinea pigs fed a diet of rat pellets and fresh kale was 75 per cent versus 37 per cent in animals fed rabbit pellets and fresh kale. It was concluded that this difference might be caused by the differences in the protein and roughage content of pellets; therefore, a diet of canned milk was used whereby the animals would get no roughage but a good source of animal protein. The infectivity rate of the guinea pigs on this diet was extremely high but the animals ate very little. Following this, numerous experiments were carried out adding various nutrients to canned milk, but the animals continued to exhibit a poor nutritional status. The MGP diet was then formulated.

A total of 186 inoculated guinea pigs, representing 21 experiments, have been maintained on this diet. Amebic lesions were found in 179 of the animals (infectivity rate of 96 per cent) and the mortality rate was also 96 per cent. The mean day of death for the 179 infected guinea pigs was 8.2 days; the greatest number of these deaths occurred between the fifth and the eleventh day. In 17 of the 21 experiments all the animals died and had amebic lesions and demonstrable trophozoites in the cecal content. The average degree of infection (ADI) of the 179 animals was 3.8 out of a possible 4.0.

Weight records on 186 infected and 28 non-infected controls fed the MGP diet are difficult to summarize because of variation in response. Among the infected guinea pigs the following patterns were observed: (1) weight gains prior to inoculations, continuous weight loss after inoculation and until death; (2) weight remained constant until a few days prior to death, then decreased rapidly; (3) weight decreased from the beginning of the experimental period until death; (4) periods of increase and decrease in weight until a few days before death when there was a rapid weight loss. In all cases, as shown above, there was a terminal weight loss.

TABLE 2

Comparison of the effects of 2 different diets on guinea pigs inoculated with E. histolytica

DIET	NUMBER OF GUINEA PIGS INOCULATED	NUMBER DEAD WITH AMEBIC INFECTIONS	MEAN DAY OF DEATH	AVERAGE DEGREE INFECTION (ADI)	PER CENT SURVIVAL FOR 30 DAYS
A. Modified guinea pig	28	27	8.4 ± 0.59*	3.9 ± 0.039†	4
B. Rat breeder	28	22	14.8 ± 1.4	3.3 ± 0.25	14

* Diet A *vs.* Diet B, difference between the means = 6.4; P <0.01.
† Diet A *vs.* Diet B, difference between ADI = 0.6; P <0.01.

TABLE 3

The effect of 3 different diets on E. histolytica *infection in the rat*

RAT PELLETS			RAT BREEDER			MODIFIED GUINEA PIG (MGP)		
No. Animals	ADI	Weight Gain (gm.)	No. Animals	ADI	Weight Gain (gm.)	No. Animals	ADI	Weight Gain (gm.)
10	0.5	18	10	3.0	11	9	2.5	1
6	1.7	22	10	3.2	20	9	3.5	5
7	0.4	19	7	2.9	21	8	3.8	1
18	1.6	19	18	4.1	16			
Mean	1.14 ± .29	19.2		3.49 ± .25	16.5		3.24 ± .32	2.4

Of the 28 control animals 13 died prior to the end of the 30-day experimental period. These controls showed similar patterns of increase and decrease in weights.

The rat breeder diet, which gave consistently high amebic infection rates in rats, was employed in three studies. In these three experiments (not included in the 21 experiments reported above) the effects of the MGP and breeder diets have been compared, using a total of 28 guinea pigs for each test diet. All but one of the guinea pigs receiving the MGP diet died with amebic lesions and demonstrable amebae, while six of the animals on the breeder ration survived the 30-day period and were found to be negative for lesions and amebae at the time they were killed. The mean day of death for the animals on the MGP diet was 8.4 ± 0.59 and for those on the breeder diet 14.8 ± 1.4. The ADI for the in-

fected animals fed the MGP ration was 3.9 ± 0.039, while that for the infected animals on the breeder ration was 3.3 ± 0.25. The means of the day of death and the ADI were both significantly different ($P < 0.01$) (Table 2).

The weight records for the animals fed the breeder diet showed the same patterns of fluctuation for controls and inoculated animals as with the other diet.

Rats

Four experiments were performed in which the rat breeder diet and commercial rat pellets (Purina) were compared both for their effect on amebiasis and on growth of the animals. In three of these experiments a group of rats was fed the MGP diet.

The mean ADI (Table 3) of the rats fed the MGP diet and of those fed the breeder diet was 3.24 ± 0.32 and 3.49 ± 0.25, respectively; that of rats fed the commercial rat pellets was 1.14 ± 0.29. The mean ADI of the first two was significantly higher than that of the last.

In spite of the difference in the degree of amebic infection, the rats fed the commercial rat pellets and those receiving the rat breeder diet gained weight at approximately the same rate (Table 3). The MGP diet failed to promote normal weight gains (Table 3).

DISCUSSION

The results of these experiments confirm in general the observations of previous investigators listed in Table 1, namely, that diet influences protozoan infections in the gut of laboratory animals.

No definite explanation can be given at this time as to why Kessel (1924, 1929) and Kessel and Huang (1926) found that milk and milk products suppressed the numbers of amebae in their experiments, and in our experiments with rats and guinea pigs a diet containing 50 per cent dry skim milk enhanced *E. histolytica* growth.

A comparison of the MGP diet and the rat breeder diet in the guinea pig and rat would indicate that some of the factors in the diet that influence amebiasis in these animals were not the same. The rat breeder diet which partially suppressed the infection in the guinea pig had no such effect in the rat. On the other hand, the MGP diet produced a relatively high rate of infection in both animals. If the factors were the same, one would expect comparable results in the two hosts.

In spite of the fact that the MGP diet in the rat was associated with a high rate of infection and a low rate of weight gain, we can exclude the possibility that a nutritional deficiency is a necessary adjunct to a high rate of infection. While neither the MGP diet nor the rat breeder diet were especially suited for growth of the guinea pig, there were no great differences in their influence on guinea pig growth. There was, however, a difference in their influence on the course of the infection in this host. Similarly, the commercial rat pellets and the rat breeder diet were equivalent in their growth promoting properties in the rat, yet they produced significantly different rates of infection.

One of the objects of these experiments was to obtain a diet which would permit a maximum rate of amebic infection in the rat and the guinea pig. The corollary problem of detailing the specific factors which influence the course of infection would best be approached by the use of completely synthetic diets. The results of such investigations, which are now under way, will be reported later.

SUMMARY

The effect of two different compounded diets on experimentally induced *Endamoeba histolytica* infection in two hosts, the rat and the guinea pig, has been studied and the results reported here.

When the guinea pigs were maintained on the modified guinea pig (MGP) diet, almost 100 per cent of the animals died of the infection with a mean day of death of 8.4 days, and an average degree of infection of 3.9 out of a possible 4.0. When the rat breeder diet was fed to the guinea pigs only 78 per cent of the animals died of the infection with the mean day of death of 14.8 days and an average degree of infection of 3.3. The differences produced by the two diets were statistically significant.

In rats both diets produced a high incidence of infection and a high average degree of infection.

The data presented here, along with the work already reported from our laboratory, add further evidence to that reported by others, that diet influences the course of amebic infection in rats and guinea pigs.

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IMMUNITY TO AMEBIC INFECTION IN DOGS[1, 2]

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This study, which represents part of a project designed to investigate the immunology of amebiasis, pertains principally to acquired immunity to amebic infection in dogs. An attempt has been made to assess the degree of resistance to reinfection with *Endamoeba histolytica* in dogs following elimination of previous amebic infection. Strains of *E. histolytica* which are not identical have been employed to determine whether acquired immunity to reinfection in dogs is homologous or heterologous in range. Further, an attempt has been initiated to determine whether or not immunity to amebic infection in dogs is passively transferable in the blood of immune animals. Previously reported studies in this project on the immunology of amebiasis dealt with induced resistance to amebic infection in rats by means of injection of amebic extracts prior to inoculation (Swartzwelder *et al.*, 1950).

STUDIES ON ACQUIRED IMMUNITY TO REINFECTION

Dogs with an estimated age of one or more years were used in this study. The series included both male and female animals in approximately equal numbers. In view of the occurrence of natural infection with *E. histolytica* in dogs, it was necessary to obtain adequate evidence of the absence of natural infection in animals prior to their use in the investigation. Since salmon will exacerbate latent amebic infection in dogs, all animals were placed on a diet of canned salmon for one week before they were included in the experimental series (Faust *et al.*, 1934). Material was aspirated from the large intestine at least three times during this dietary period and examined microscopically for the presence of *E. histolytica*. Additional diagnostic aspirations were done on all animals before they were exposed to inoculation. Following the week on a salmon diet, the dogs were placed on a diet of Purina Dog Chow Checkers.

The animals were inoculated by the introduction *per anum* of mucosanguineous material containing trophozoites which had been aspirated from source animals with chronic amebic dysentery (Faust, 1931). Each inoculum was derived and pooled from several source animals. On a few occasions the inocula were augmented by trophozoites from cultures of the same strain in order to increase the number of organisms. Animals were not fed on the day before nor on the day of inoculation. Otherwise, the diet immediately prior to and after inoculation consisted of the dog chow checkers. No enemas were employed as preparation for inoculation. The dogs were placed under Nembutal anesthesia during the inocu-

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lation in order to minimize the risk of expelling the inocula. Introduction of inocula was by means of a long glass tube with a rubber bulb. Inocula were delivered into the bowel from twelve to fifteen inches above the anus. The dogs were inverted at a 45 degree angle during inoculation and maintained in this position for one hour after the procedure. The dogs were inoculated with only one strain at a time.

Following inoculation of the dogs, colonic aspirates and stools were checked daily until the infection became established. After that, two or three examinations per week sufficed. The aspirates were examined macroscopically for gross blood, and microscopically for the presence of *E. histolytica* and of blood. The stools were examined for gross blood.

Cultures of aspirates from the large intestine of all animals for bacterial pathogens were made prior to inoculation and at weekly intervals during the study. The aspirates were plated on S-S and MacConkey's media. This was done as a control measure to detect any pathogenic bacteria which might complicate and alter the results of the investigation. None was found.

At first, the initial infections in some animals were terminated by chemotherapy. The drugs employed were emetine hydrochloride, chiniofon (Anayodin) and diiodohydroxyquinoline (Diodoquin). It was observed, however, that infection usually would terminate spontaneously if allowed to run its course. In view of this observation, the use of chemotherapy was abandoned.

Particular care was taken to rule out the presence of persistent infection after chemotherapy or after apparent spontaneous cure. In this way reactivation of latent infection could not be confused with reinfection. After the initial infection was terminated and exhaustive examinations of aspirates for several weeks or months revealed no evidence of *E. histolytica*, and a salmon diet failed to elicit evidence of latent amebic infection, the dogs were subjected to reinoculation with the same strain. If the same strain could not be reestablished in the dog on the first reinoculation, another strain was employed for two or more inoculations. Occasionally the order in which the strains were employed was varied during the four inoculations. If no evidence of reinfection resulted within one month of each reinoculation, the salmon diet technique and daily aspiration of the large bowel for several weeks were again instituted to determine the presence or absence of latent infection. Most animals were subjected to four inoculations. A few animals received three or five inoculations. The dogs were sacrificed and autopsied after the final unsuccessful attempt to reinfect them. By this means the absence of infection could be determined with certainty. Latent amebic infection was never demonstrated at autopsy when the aspirates had been negative for *E. histolytica* during a test salmon diet.

The number of trophozoites in an inoculum was computed from counts of a sample of the pooled dysenteric aspirates. It was necessary to employ a dilution technique on the sample, due to its mucosanguineous consistency, in order to permit detection and counting of the trophozoites. The minimum number of trophozoites of the two strains employed which produced infection was 115,000 and 189,500. An attempt was made to use approximately the same number of trophozoites on the first reinoculation as was employed in the initial inoculation.

The number of trophozoites in the inocula was increased, when possible, for the subsequent reinoculations. Many animals were exposed to over 1,000,000 trophozoites during reinoculation. The maximum number of trophozoites in the inocula employed was 2,486,000. The animal in this case was completely refractory to reinfection with this inoculum.

Natural infection with amebiasis was frequently encountered in the dogs received for experimental use. Two natural infection strains, A and H, were selected for use in the study. The strains did not cause a high mortality rate, yet they produced infections long and severe enough to provide criteria for comparison of initial infection and subsequent infections if resistance to reinfection did not prove to be absolute. The amebic strains were obtained from source animals two years apart. The amebae from these two sources differed distinctly in their culturability. Strain H could be cultured with ease. Despite repeated attempts, strain A could not be cultured at all on two media—egg slant with buffered saline overlay and Cleveland and Collier's medium. These media were used to cultivate strain H. Other media were also employed at another laboratory in an attempt to culture strain A, without success. Faust and his associates (1946) have presented evidence which indicates that culturability, infectivity and pathogenicity are three separate characteristics of *E. histolytica*. In iron-hematoxylin stained preparations the morphology of the trophozoites of both strains was compatible with that of *E. histolytica* trophozoites of human origin. The strains produced a dysentery and typical amebic lesions in cats and rats. The above findings are similar to established experience with strains of *E. histolytica* of human origin in experimental hosts. Based on the difference in culturability of the amebae from the two source animals, it was concluded that strains A and H were distinct entities. This factor was of significance, since it provided a means of measuring the degree of strain specificity of any immunity which might be demonstrated.

Complement fixation tests were not done on these animals. The time required for hundreds of inoculations and several thousand microscopic examinations did not permit the undertaking of extensive serological testing. The criteria for determining the presence or absence of amebic infection which were employed proved to be adequate, without supplemental serological evidence. Further, complement fixing antibodies do not persist long in dogs after termination of infections (Craig, 1944). Therefore, they would have provided no direct or indirect evidence of persistence of immunity. Direct evidence of persistence of immunity was actually demonstrated by the technique employed in these studies. The inability to demonstrate precipitins in dogs infected with *E. histolytica* were reported previously (Swartzwelder *et al.*, 1949).

RESULTS

Amebic infection was established in 39 of 46 series animals, with either strain A or H, on first inoculation. Infection failed to become established in 7, or 15 per cent, of these dogs. The initial induced infection rate was 85 per cent. None of the animals in this series received a pre-inoculation transfusion.

Twenty-nine (29) animals which developed amebic infection as a result of

their initial inoculation were reinoculated from two to four times with challenge doses equivalent to or greater than the initial inoculum. Despite repeated attempts to reestablish infections in these animals, using large inocula, only 5 of the 29 dogs could be infected. The reinfection rate was 17 per cent. About 83 per cent of the dogs initially infected became completely refractory to reinfection. The results of the reinoculations were the converse of those obtained from the initial inoculations.

TABLE 1

Initial infection rate versus reinfection rate

Infection rate from initial inoculation:	85%
Reinfection rate (in dogs previously infected):	17%
Refractory to infection from initial inoculation:	15%
Refractory to reinfection (in dogs previously infected)	83%
Initial Infection Rate:	85%
versus	
Reinfection Rate:	17%

The pattern of the initial infections and reinfections of the 5 animals which could be reinfected appeared to indicate the presence of some immunity to reinfection in two of the dogs. However, there was no marked increase in immunity with each reinfection in any of these five animals. It was possible to reinfect some of these non-resistant animals repeatedly.

It was felt that amebae must be detectable for at least one week after inoculation or reinoculation to consider an infection as established. In a few reinoculated animals, trophozoites were detected one or two days after inoculation. However the infection was unable to become established in the intestine of the animals. In some of these cases, the trophozoites probably represented part of the inoculum. In a few other animals, these transient episodes probably represented infections which were aborted before they could become well established.

Two examples of representative records of series animals are presented below:

Dog No. 25

Sex: Female Range of weight: 12 to 16 lbs.

INOC. NO.	DATE	STRAIN	NO. OF TROPHS. IN INOCULA	PREPATENT PERIOD	FIRST DAY		
					GROSS BLOOD IN STOOL	GROSS BLOOD IN ASPIRATE	MICROSCOPIC BLOOD IN ASPIRATE
(1)	12/2/48	H	400,000	4 days	4	4	4
(2)	8/9/49	H	420,000	Neg.	Neg.	Neg.	Neg.
(3)	9/13/49	A	280,000	Neg.	Neg.	Neg.	Neg.
(4)	10/11/49	A	414,000	Neg.	Neg.	Neg.	Neg.

Duration of initial infection: 5 months
Termination of initial infection: spontaneous
Tested duration of resistance to reinfection: 5 months
Number of challenge inoculations: 3 (1, homologous strain; 2, heterologous strain)
Resistance to reinfection: complete
Sacrificed 2/14/50: bowel negative for amebae

Dog No. 34

Sex: Male Range of weight: 23 to 26 lbs.

INOC. NO.	DATE	STRAIN	NO. OF TROPHS. IN INOCULA	PREPATENT PERIOD	FIRST DAY		
					GROSS BLOOD IN STOOL	GROSS BLOOD IN ASPIRATE	MICROSCOPIC BLOOD IN ASPIRATE
(1)	12/16/48	H	399,750	11 days	12	12	12
(2)	2/22/49	A	407,000	Neg.	Neg.	Neg.	Neg.
(3)	4/26/49	A	1,260,000	Neg.	Neg.	Neg.	Neg.
(4)	7/20/49	H	648,000	Neg.	Neg.	Neg.	Neg.

Duration of initial infection: 1 month
Termination of initial infection: spontaneous
Tested duration of resistance to reinfection: 6 months
Number of challenge inoculations: 3 (1, homologous strain; 2, heterologous strain)
Resistance to reinfection: complete
Sacrificed 8/31/49: bowel negative for amebae

Of the 24 dogs which were refractory to reinfection, 23 were tested for immunity to an heterologous strain. All 23 were refractory to infection with the heterologous strain. All 24 animals were challenged for immunity to the homologous strain with which they were infected originally. All 24 dogs were refractory to reinfection with the homologous strain. In other words, of the 24 dogs that became completely refractory to reinfection following a single initial infection, 23 were challenged with inocula of both strains, one at a time. All 23 animals were refractory to reinfection with either strain. The resistance to reinfection with amebiasis in dogs was clearly active against both homologous and heterologous strains of *Endamoeba histolytica*.

The tested duration of immunity to reinfection (*i.e.*, from the time of eradication or loss of the initial infection to the date of the last challenge inoculation which failed to induce reinfection) ranged from 10 weeks to nine and one-half months. The immunity was challenged over a period of six months in a majority of refractory animals. Over the periods observed, no animal lost its resistance to reinfection once it was established following an initial infection. In view of this observation, it was concluded that the resistance to reinfection probably persisted much longer than the maximum period of nine and one-half months during which the immunity was challenged in these studies.

There was no apparent correlation between the duration of initial infection and the presence or absence of resistance to reinfection. Nor was there any correlation between the mode of termination of the initial infection, *i.e.*, by chemotherapy versus spontaneous recovery, and the presence or absence of resistance to reinfection. Further, resistance to reinfection with amebiasis in dogs did not depend upon the presence of latent infection with the parasite.

Although it would have been desirable and ideal to employ dogs raised from birth without any exposure to amebiasis in nature, this was not practicable. During the course of these experimental studies, 70 dogs were received and examined to detect the presence of absence of natural infection. Seven dogs were found to harbor natural amebic infection at the time of procurement. A few of

these seven animals had dysentery at the time of receipt. The others developed a dysentery when placed routinely on a salmon diet prior to experimental use in order to exacerbate any possible latent natural amebic infection. This represented a 10 per cent natural infection rate of amebiasis in dogs. As previously indicated, infection could not be induced in 15 per cent of the experimental animals which were proved to be ameba-free before inclusion in the experimental series. Since 10 per cent of all animals which were received harbored natural infections, the possibility exists that the ameba-free dogs which could not be infected at all despite multiple inoculations (15 per cent) may have recovered from natural infections acquired before they were procured for experimental purposes.

Eight additional natural infections were detected in dogs procured for other departments at the medical school. Thus, a total of 15 natural amebic infections in dogs was found. Attempts were made to culture amebae from eight of these infections. Three produced luxuriant continued growth on egg slant media overlayed with buffered saline. Trophozoites from two other naturally infected dogs persisted in cultures only through a few transfers and three failed to grow in culture after one transfer.

TABLE 2

Possible relationship of natural infection to resistance to experimental infection

Natural infection rate:	10%
Refractory to infection from initial inoculation:	15%

Studies on passive transfer of immunity in amebiasis

This study was initiated to determine whether or not passive transfer of immunity in amebiasis in dogs was possible by means of blood transfusions. Fifty and 100 cc. volumes of blood were removed from dogs weighing from 5 to 10 kilos. An equivalent volume of blood was transfered to these animals from donor dogs which had become completely refractory to reinfection with strains A and H. Within two to five days these animals were given their first inoculation. If the dog did not develop amebic infection, as indicated by the salmon diet technique and repeated colonic aspirations, they were reinoculated. A comparison of the infection rate of all series animals which received no transfusions with that of animals which received preinoculation transfusions was used to obtain a preliminary indication of the possibility of passive transfer of immunity in amebiasis by means of blood transfusion.

RESULTS

Definite conclusions cannot be drawn from the study initiated on the possibility of passive transfer of immunity to amebiasis in dogs by means of blood transfusion. Only ten animals were used and the results were not as sharply defined as in the case of the study on naturally acquired resistance to reinfection. However, based upon the results of this initial limited exploration of the possibility of passive transfer of immunity in amebiasis, certain indications are apparent.

The initial infection rate, based upon a single inoculation with heavy inoculum, was 30 per cent. It was possible to induce infection in three additional animals by a second inoculation one to three months after the initial pre-inoculation transfusion. This delay in reinoculation was necessary in order to exclude, by salmon diet and repeated colonic aspirations, the presence of infection from the initial inoculation. This increased the infection rate to 60 per cent based upon two inoculations. The remaining animals (40 per cent) could not be infected either within a few days after transfusion or from one to three months later.

The differences in the infection rates and failure rates between series animals which receive no pre-inoculation transfusion and the dogs which were transfused with blood of ameba-resistant donor dogs is shown in Table 3.

TABLE 3

Comparison of infection rates in dogs with *versus* without *pre-inoculation blood transfusion from immune animals*

	INFECTION RATE	FAILURE RATE
Series dogs (no pre-inoculation transfusion)		
Initial inoculation	85%	15%
Dogs transfused prior to inoculation		
Initial inoculation	30%	70%
Second inoulcation	60%	40%

DISCUSSION

Thompson and Lilligren (1949) reported that untreated dogs, even on a fish diet, developed an increased resistance to *Endamoeba histolytica* which was manifested by the development of subacute infection from a previously acute form of the disease. They were able to reinfect four out of five dogs following chemotherapy. However, in each instance reinfection was accomplished only after two or three reinoculations. The number of organisms in each inoculum was not determined, but the material was similar in volume to that which regularly produced infection in dogs that had not been infected previously. The one animal which could not be reinfected was inoculated with large numbers of infective amebae on four occasions over a period of four months. This animal, following a recovery from its initial infection, had become completely refractory to *E. histolytica* in spite of maintenance on a fish diet.

These observations of Thompson and Lilligren were incidental to a study on the chemotherapy of experimental amebiasis. Their report on the immunology was based upon a much smaller series of animals than ours. Their results appear to indicate, however, the definite presence of a degree of acquired immunity to reinfection. This immunity seems to have been less marked than that observed by us in our larger series. The difference in the results of these two studies is not one of the presence or absence of acquired immunity in dogs, but lies in the degree of immunity acquired.

Spontaneous recovery of dogs from experimental amebiasis was reported by Faust (1932) almost two decades ago. This phenomenon was very frequently

observed by the authors to occur in dogs after a few or several months of infection in this study. The occurrence of spontaneous recovery after long established infection is a phenomenon which suggests the development of an immunity. In contrast, however, an occasional dog has remained infected with the same strain for several years. In some dogs, spontaneous recovery from experimental amebiasis apparently may be postponed and chronic or acute infections may be prolonged by feeding a salmon diet.

Wagner (1935) is reported to have obtained evidence that a degree of immunity to reinfection is produced in cats after two or three separate infections. In dogs, Wagner is reported to have found little indication of such an acquired immunity. His results, obtained as observations incidental to work undertaken primarily with the object of chemotherapeutic studies on amebic dysentery, are at variance with those of the authors. The present study was carefully controlled and conducted with precise attention to detail and with the primary object of studying the immunology of experimental amebiasis in dogs.

The statement that immunity after recovery from amebic infection appears to last only as long as the causal parasites persist as a latent form of infection in the body of the patient (Culbertson, 1941) certainly did not obtain in dogs in the present study. However, it is not claimed that the results observed in these experiments in which dogs were employed would necessarily obtain in humans.

The authors were unable to find any work in experimental amebiasis relating to the range of immunity (homologous versus heterologous) and thus no comparison of their results with other work in this phase of the subject is possible. Similarly, the passive transfer of immunity to amebic infection via the sera or blood of recovered animals apparently has not been explored previously.

SUMMARY

An attempt has been made to assess the degree of resistance to reinfection with *Endamoeba histolytica* following elimination of previous amebic infection. Inoculation was made by intubation *per anum* of trophozoites in dysenteric aspirates obtained from infected dogs.

The initial induced infection rate of amebiasis in dogs was 85 per cent. Despite repeated attempts to reestablish infections in animals whose initial infection had been terminated, using equivalent or greater inocula, only 17 per cent could be reinfected. This indicates the development of acquired immunity to reinfection with *E. histolytica* in dogs.

The resistance to reinfection in dogs was active against both homologous and heterologous strains of *E. histolytica*. The strains employed differed distinctly in their culturability.

The tested duration of immunity to reinfection ranged from ten weeks to 9½ months. Over the periods observed, no animal lost its resistance to reinfection once it was established following an initial infection.

A small series of animals which received pre-inoculation blood transfusions from dogs which had become refractory to reinfection with *E. histolytica*, thus far has shown a lower infection rate than that of animals which were not trans-

fused. This suggests the possibility of passive transfer of immunity in experimental amebiasis in dogs.

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STUDIES ON TREATMENT OF FRESH VEGETABLES CONTAMINATED WITH CYSTS OF *ENDAMOEBA HISTOLYTICA*. I. ACETIC ACID

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It is believed that under some circumstances contaminated fruits and vegetables may be a factor in the spread of amebiasis. Consequently, methods used to treat such foods should be effective for the destruction of cysts of *Endamoeba histolytica* as well as other pathogenic organisms. Various treatments have been suggested but none has been wholly satisfactory, especially for leafy vegetables. To evaluate a treatment for this purpose data are necessary as to its efficacy against cysts in suspension and also against cysts actually present on the foods treated. Observations on vegetables, not only immediately after treatment but also after some hours of storage, also are pertinent.

The cysticidal efficacy of acetic acid, long recognized as having bactericidal and bacteriostatic properties, was first noted by Beaver and Deschamps (1949). They recommended that vegetables be immersed for 15 minutes in a 5 per cent solution for the destruction of *E. histolytica* cysts.

Additional information regarding the use of acetic acid for the treatment of vegetables contaminated with cysts of *E. histolytica* was considered desirable. Of special interest was the possibility of using less concentrated solutions of acid and shorter contact periods by increasing the temperature during treatment. The temperature factor has been emphasized in the present study. Data obtained from experiments with cysts suspended in solutions of the acid have been supplemented with data obtained from the treatment of cysts present as (laboratory) contaminations on vegetables. Observations were made also on a variety of vegetables after contact with various concentrations of acid at several temperatures.

MATERIALS AND METHODS

Cysts of the NRS strain of *E. histolytica*, obtained from culture, were used. They were concentrated and washed by centrifugation in distilled water and stored at 5°C. They were used 1 to 4 days (usually 1 day) after preparation. The number of cysts was estimated by counting several 0.05 ml. samples on a ruled slide. Constant temperatures were maintained during the experiments by means of a thermostatically controlled water bath.

Aside from the acetic acid used in most tests, a commercial vinegar was employed in a few. This was a white distilled vinegar of 5 per cent acidity.

Not all varieties of vegetables were treated with all the combinations of acid, temperature, and time of exposure. Most tests were with leafy garden lettuce, head lettuce, parsley, water cress, celery, and carrots. Some observations were

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also made on cabbage, celery cabbage, endive, radishes, and tomatoes. The procedures in the experiments with cysts alone, with a few exceptions, were as follows: To 10 ml. of acetic acid, twice the desired strength, were added distilled water and (as soon as the temperature was stabilized) the concentrated cyst suspension in such amounts as to provide 2000 cysts per ml. of acid solution of the desired concentration. This mixture was stirred with a thermometer calibrated to 0.1° C, and temperatures were read every 15 to 30 seconds. At the end of the exposure period, the treated material was diluted rapidly with an equal volume of chilled, distilled water containing sodium bicarbonate, in order to reduce the temperature and to neutralize the acid. Part of the material was then inoculated directly into 5 tubes of medium at 1000 cysts per tube and after suitable dilutions of the cyst suspension, 5 tubes were inoculated with 100, and 5 with 10 cysts per tube. The remainder of the treated material was centrifuged and washed 2 or 3 times. The number of cysts was estimated by counting several 0.05 ml. samples, and with appropriate dilution, these washed cysts were similarly inoculated into 15 tubes of medium. In some instances any remaining material was concentrated by centrifugation and inoculated into 1 or 2 tubes.

Routinely, controls on the cyst lot employed in each experiment were represented by 15 cultures, 5 inoculated with 20 cysts per tube, 5 with 10, and 5 with 5 cysts per tube. In addition, 5 control cultures with 10 cysts per tube were set up in order to check growth in medium containing amounts of neutralized acid (i.e. sodium acetate) comparable to the maximum amount inoculated into cultures with the treated cysts in any given experiment.

In experiments concerned with treatment of contaminated vegetables, concentrated cyst suspensions were put directly on small fragments of vegetables, or before application, were mixed with a commercial "potting soil" which had been autoclaved before use. In early tests the contaminated vegetable samples were inoculated into medium directly after treatment or after rinsing to reduce temperature and acidity. It was believed that these tests should give some indication as to whether cysts actually on vegetables would be protected from action of the acid. However, since the numbers of cysts which adhered to the vegetables after treatment and were inoculated were not definitely known, the tests were considered to be of limited value and later another technique was employed.

In the later experiments, larger samples of lettuce were contaminated with known quantities of concentrated cyst suspensions. After treatment with acetic acid the lettuce was immersed in a soda water rinse; then lettuce and rinse were blended for two minutes in a Waring blender; ice was added to maintain a low temperature when necessary. The blended material was then strained through one layer of gauze. The filtrate was centrifuged, cysts were counted in 0.05 ml. samples of the sediment, and inoculations into medium were made, the numbers of cultures and of cysts per tube varying with the numbers of cysts recovered. Preliminary tests with this technique indicated that at least 65 to 70 per cent of cysts applied to lettuce before blending could be recovered in the filtrate and also that the presence of the blended lettuce did not adversely affect growth in culture of untreated cysts. Samples of each head of lettuce used were similarly

blended and inoculated into medium to serve as a control against unknown amebic contamination of the vegetable.

L.E.R. medium, a modified Boeck-Drbohlav medium (Reardon *et al.*, 1939) was used routinely. In some experiments, additional inoculations were made into Balamuth and Sandza's all liquid medium (1944), but it appeared to have no advantages for this material and its use was not continued. Bacteria from the NRS strain cultures were added to all cultures. Cultures were examined on the third day of incubation and, if negative, subcultures were made. Three days later both the originals and the subcultures were examined.

TABLE 1

Results of inoculations into culture medium of Endamoeba histolytica *cysts after 5 minutes exposure to acetic acid of 3 concentrations at 25° to 45°C.*

	25°	30°	35°	40°	45°
1.0 per cent acid	5 5 5*	5 5 3	5 5 3	5 3 0	0 0 0
				5 2 0	0 0 0
				2 0 0	0 0 0
				2 1 0	
Estimated per cent destruction	?	<90	<90	>90	100
2.5 per cent acid	5 5 4	5 5 0	5 4 1	0 0	0 0 0
		5 5 0	5 2 0	0 0	0 0 0
		5 5 3	4 2 0	0 0	0 0 0
		5 5 2	5 4 1		0 0 0
					0 0 0
					0 0 0
Estimated per cent destruction	<90	<90	90±	100	100
5.0 per cent acid	5 5 4	5 1 0	0 0 0	0 0	0 0 0
	5 5 2	4 0 0	0 0 0	0 0	0 0 0
		2 0 0	0 0 0		
		5 1 0	1 0 0		
		4 1 0	0 0		
		4 1			
Estimated per cent destruction	<90	>90	99 to 100	100	100

* The three digits represent the number of positive cultures of 15, 5 inoculated with 1,000 cysts, 5 with 100, and 5 with 10 cysts per culture tube, respectively. In a few instances the set with 10 cysts per culture was omitted. The original cyst concentration in acid was 2,000 per ml.

RESULTS AND DISCUSSION

Growth results obtained with the untreated control cysts can be summarized briefly. With the cysts which were inoculated as a suspension in distilled water, the relative numbers of positive cultures obtained were similar to those obtained with untreated cysts of this strain of *E. histolytica* in previous studies in this laboratory (Newton *et al.*, 1949; Jones *et. al.*, 1950). All cultures inoculated with 20 cysts per tube of medium were positive. Most of the cultures with an estimated 10 cysts per tube were positive, with an average of 4.8 positive tubes for each set of 5 inoculated; with 5 cysts per tube an average of 4.3 tubes positive for each set of 5 inoculated was obtained.

Additional controls were set up in view of the possibility that the sodium acetate which resulted from neutralization of the acid in the cyst suspension might affect growth in culture. These controls provided information as to growth in 5 cultures, each inoculated with 10 untreated cysts, under conditions for growth comparable with the maximum neutralized acid concentration in the experimental series inoculated directly without washing or dilution. In the experiments employing 1 per cent and 2.5 per cent neutralized acid, growth was very similar to that obtained from inocula of cysts suspended in distilled water, the average number of positive cultures being 4.9 in both of the neutralized acid series. With 5 per cent neutralized acid, the average number of positive cultures was 4.4 of 5 inoculated.

The method of estimating percentage of cyst destruction has been reported previously (Newton *et al.*, 1949; Jones *et al.*, 1950). Less than 5 positive cultures of 5 inoculated is considered indicative of the presence of less than 10 undamaged cysts per tube.

In table 1 are presented growth results obtained after cysts were exposed for 5 minutes to 1 per cent, 2.5 per cent, and 5 per cent acetic acid at temperatures of 25°, 30°, 35°, 40°, and 45°C. The increased cyst destruction by 5 minutes contact, resulting from increased acid concentration is evident at temperatures of 30°, 35°, and 40°C. The influence of temperature on cyst destruction by each of the concentrations of acid is also evident. What may be termed a critical temperature range for 5 minutes exposure is 35° to 45° for a 1 per cent solution, 30° to 40° for a 2.5 per cent solution, and 25° to 35°C. for a 5 per cent solution of acetic acid.

The estimated percentages of cyst destruction after 5 minutes exposure to the 3 concentrations of acetic acid and the temperatures noted above also are included in table 1. More than 90 per cent cyst destruction occurred after 5 minutes exposure to 1 per cent acid at 40°C., to 2.5 per cent acid at 35°C., and to 5 per cent acid at 30°C. To achieve 99 to 100 per cent cyst destruction under these conditions, temperatures of 45°C., 40°C., and 35°C. are indicated with acetic acid concentrations of 1 per cent, 2.5 per cent, and 5 per cent, respectively.

In table 2 data are presented on results obtained after cysts were exposed for various time intervals to 5 per cent acetic acid at 25°C. and 30°C., to 2.5 per cent acid at 35° and 45°C., and to 1 per cent acid at 50°C. It will be noted that complete cyst destruction was not obtained by exposure for 15 minutes to 5 per cent acid at 25°C. However, at 30°C. complete cyst destruction was evident. Additional evidence of this destruction, not included in the table, was provided in 1 experiment by negative results in a series of 5 cultures each inoculated with 10,000 cysts. A recommendation of 15 minutes treatment with 5 per cent acetic acid apparently should include mention of a temperature of at least 30°C.

Considerable, although not complete cyst destruction was obtained by exposure to 2.5 per cent acetic acid for 10 minutes at 35°C. The effects of longer exposures to these conditions were not determined. In experiments with 2.5 per cent acid at 45°C., complete cyst destruction was evident after exposures of only 3 or 5 minutes. The data indicate that 5 minutes immersion of vegetables

in a 2.5 per cent acetic solution at 45°C. might be a reasonable recommendation as far as cysts of *E. histolytica* are concerned.

The possibility of using an even weaker solution of acid at a higher temperature was explored further by employing 1 per cent acid at 50°C., with exposures of 1 to 3 minutes. Although early tests indicated complete cyst destruction at 2 minutes, in later tests a few cysts survived this exposure. Limited observations

TABLE 2

Growth in culture after exposure of cysts of Endamoeba histolytica *to 3 concentrations of acid for various time intervals at temperatures between 25° and 50°C*

ACETIC ACID	TEMP.	MINUTES EXPOSURES					
		1	2	3	5	10	15
%	°C.						
5.0	25°	5 5*			5 5		5 1
							4 0
	30°				5 1		
					4 0		
					4 1	1 0	0 0
						1 0	0 0
2.5	35°	5 5			5 2	2 0	
						0 0	
	45°				0 0		
					0 0		
					0 0		
					0 0		
		5 0		0 0	0 0		
					0 0		
		1 0		0 0			
		1 0		0 0			
				0 0			
1.0	50°	1 0	0 0				
			0 0				
			0 0				
			1 0				
		1 0		0 0			

* Number positive cultures of 5 inoculated with 1,000 cysts per tube and 100 cysts per tube, respectively.

indicated complete destruction after 3 minutes exposure. It was concluded however that although 3 minutes exposure to 1 per cent acetic acid at 50°C. should be effective, such treatment of vegetables would provide little margin of safety and from a practical standpoint would be less desirable than treatments just discussed.

A further reason for the interest in acetic acid as a rinse for vegetables is the possibility of substituting vinegar which is easily obtainable and also generally acceptable on foods. Beaver and Deschamps (1949) discussed this matter and

reported that in tests with local vinegar, one variety failed to kill cysts within 15 or 20 minutes and another variety failed with an exposure of 1½ hours. However they state that definite conclusions regarding the relative value of vinegars as compared with acetic acid of equal strength await further observations.

The particular undiluted vinegar used in the present study failed to destroy cysts (250 cysts per ml.) in 15 minutes at 25°C. At 45°C. cysts were destroyed by 3 minutes exposure to one-half strength vinegar comparable to 2.5 per cent acetic acid; cysts concentrations were 1,000 per ml. in 1 test and 2,000 per ml. in 3 tests. Cysts were killed in 2 tests by exposure at 50°C. for 2 minutes to vinegar of one-fifth strength, comparable to 1 per cent acetic acid. Although there was some evidence of a slightly higher cyst survival with undiluted vinegar than with 5 per cent acetic acid at 25°C., at the higher temperatures no differences were noted between the vinegar tested and pure acetic acid.

Most of the observations of the effect of acetic acid on vegetables were made after treatments for 15 minutes in 5 per cent acid at 25°C., 5 minutes in 2.5 per cent acid at 45°C., and 3 minutes in 1 per cent acid at 50°C. After exposures to these conditions, carrots, celery stalks, parsley, water cress, head lettuce, and garden lettuce were acceptable as to taste and appearance immediately after treatment. Carrots, celery, and parsley were still acceptable 16 to 24 hours later (with overnight refrigeration). Some head lettuce was usable 2 to 4 hours after treatment, but not after 24 hours, except for some parts of the samples exposed for 3 minutes to 1 per cent acid at 50°C. Some of the water cress was still usable after 2 to 4 hours but very little of it by 24 hours. Leafy garden lettuce, as might be expected, was less resistant; very little of it was acceptable 2 to 4 hours after treatment and none of it after 24 hours.

Tomatoes were not affected by 5 minutes exposure at 45 to 46°C. to either 2.5 per cent acetic acid or half-strength vinegar. Observations on other vegetables after exposure for 5 minutes to 2.5 per cent acid at 45°C. indicated that cabbage was acceptable up to 24 hours after treatment; part but not all of celery cabbage samples were acceptable for that long; curly endive was not acceptable at 2 to 4 hours after treatment; radishes remained acceptable as to taste and texture for 24 hours although the color leached out.

A few observations were made also on carrots, celery, and leafy lettuce after 15 minutes exposure to 5 per cent acid at 30°C. All were acceptable at once, providing that care was taken that the lettuce was not unduly bruised. Carrots remained acceptable 24 hours as did most of the celery. The lettuce was usable 2 to 4 hours after treatment but not after 24 hours.

The more succulent vegetables tend to acquire an acid taste, but this is not considered undesirable, especially if they are intended for use in mixed salads. Resistance to treatment depends somewhat on the freshness of the vegetable when treated and also on the varieties, especially of the more tender greens such as lettuce.

In the earlier tests with cysts on vegetables, it was established that positive cultures could be obtained by inoculating untreated fragments of the following vegetables after they had been contaminated with *E. histolytica* cysts or with

cysts and dirt: cabbage, carrots, celery, chive, chicory, cress, lettuce, mustard, and parsley. Some positive cultures, although relatively fewer, were obtained when such vegetable fragments were rinsed by dipping in distilled water or an acetic acid and soda rinse before inoculation. These observations indicated that the presence of fragments of a variety of vegetables or of vegetables and dirt did not noticeably inhibit growth of amoebae and that if acid treatment failed to destroy the cysts positive cultures could be expected.

Results of the early experiments with contaminated vegetables treated with acetic acid are presented in table 3; only experiments in which untreated frag-

TABLE 3

Results obtained by inoculating fragments of vegetables contaminated with Endamoeba histolytica *cysts after treatment with acetic acid*

CONCENTRATION ACETIC ACID OR VINEGAR	TEMP.	TIME	CONTAMINATED VEGETABLE	GROWTH IN CULTURE*		
	°C.	*minutes*				
5 per cent acetic	25	15	lettuce	0/1		
			parsley	1/2		
			parsley	1/2		
2.5 per cent acetic	45	3	carrots	0/5		
vinegar, ½ strength	45	3	carrots	0/2		
			celery	0/2		
			cress	0/3		
			cabbage	0/2		
				1 min.	2 min.	3 min.
1 per cent acetic	50	1, 2, 3	carrots	2/5		0/5
			chicory	2/2	0/1	
			cress	0/1	0/1	
			lettuce	0/1	1/1	
			parsley	0/1	0/1	
			mustard		0/1	

* Number of positive cultures over total number inoculated.

ments gave positive cultures are included. The data are limited, but are similar to those from experiments with cysts in suspension. Partial but not complete cyst destruction occurred after treatment with 5 per cent acid for 15 minutes at 25°C. No cyst survival was evident after treatment with either 2.5 per cent acetic acid or half-strength vinegar for 3 minutes at 45°C. There was evidence of some cyst survival after 1 and 2 minutes exposure, but not after 3 minutes exposure to 1 per cent acid at 50°C.

Lettuce was the only vegetable treated in the later experiments using the blending technique. Exposures were for 15 minutes in 5 per cent acetic acid at 30°C. and for 5 minutes in 2.5 per cent acid at 45°C. The numbers of cysts recovered in the blended lettuce filtrates varied and consequently the numbers of cultures and the number of cysts inoculated per tube of medium were not

alike in all experiments. In tabulating results (see table 4) the range in number of cysts inoculated, rather than actual estimated number of cysts per tube have been used. In most instances the "mash," i.e. the blended lettuce remaining on the gauze was also inoculated but no estimates of the number of cysts in this material were attempted. In 2 of 3 experiments there was evidence of some cyst survival after contaminated lettuce had been treated with 5 per cent acetic acid for 15 minutes at 30°C. After 5 minutes exposure to 2.5 per cent acid at 45°C. there was evidence of cyst survival in only 1 of 5 experiments, and this apparently represented a low percentage of viable cysts. The estimated recovery of the original cyst contamination on the lettuce after treatment was probably not more than 5 per cent, being estimated as 1 to 4 per cent in the filtrates. No positive

TABLE 4

Growth of Endamoeba histolytica *cysts inoculated with blended lettuce after treatment with acetic acid*

ACETIC ACID	TEMPERATURE	EXPOSURE	FILTRATE			MASH	ESTIMATED CYST RECOVERY WITH LETTUCE
			Number cysts per tube medium				
			1000– to 600	550 to 350	300 to 100		
%	°C.	*minutes*					%
5	30°	15				1/7*	—
			1/7		0/3	1/6	2
					0/6	—	2
2.5	45°	5	0/2	0/1	0/3	0/6	2
			0/3		0/3	0/5	3
			0/2	0/1	0/3	0/5	3
					1/5†	—	1
				0/4	0/5‡	—	4

* Number positive cultures over number inoculated.
† Growth after 1 minute exposure 3/3.
‡ Growth after 1 minute exposure 3/4.

cultures were obtained from filtrates or "mash" of blended samples of the lettuce, which had not been contaminated with cysts.

Results of these experiments indicate that conditions sufficient for the destruction of cysts in suspensions may not be adequate for cysts present on vegetables, although with acetic acid at 45°C. the distinction was not marked.

There are some evident disadvantages to the blending procedure and, as used in these experiments, it is not well standardized as yet. In general, the volume of acid solution, the bulk of vegetables, the amount of cyst contamination, and the time interval between application of cysts to the vegetables and their subsequent treatment are all variables which bear on the extent of cyst removal by treatment and the numbers of cysts available for inoculation post-treatment. However, the use of this blending technique appears to provide a more critical evaluation of potential "germicidal rinses" for vegetables against cysts of *E. histolytica* than hitherto available.

SUMMARY

The cysticidal efficacy of acetic acid against cysts of the NRS strain of *Endamoeba histolytica* has been studied at temperatures between 25°C. and 50°C. with acid concentrations of 1 per cent, 2.5 per cent, and 5 per cent. The importance of the temperature factor has been demonstrated.

Evaluation of the acetic acid in tests against cysts in suspensions indicated the potential value as a cysticidal procedure of 15 minutes exposure to 5 per cent acid at 30°C. and of 5 minutes exposure to 2.5 per cent acid at 45°C. No cyst survival was evident under these conditions. One brand of vinegar tested gave similar results at the higher temperatures.

The importance of checking the survival of cysts actually remaining on vegetables after such treatment has been demonstrated. By a procedure of blending cyst-contaminated, treated lettuce in a Waring blender, estimates were made of numbers of cysts remaining on lettuce after treatment, and their viability was tested by inoculating into medium the filtrates and "mash" of the blended lettuce. Such experiments indicated that a few viable cysts remained on lettuce in 2 of 3 tests after 15 minutes exposure to 5 per cent acid at 30°C. and in 1 of 5 tests after 5 minutes exposure to 2.5 per cent acid at 45°C. In the latter case especially, the percentage of cysts surviving was apparently very low.

The treatment removed the greater part of the original cyst contamination from lettuce. The usefulness of the procedure is limited by the fact that some vegetables, especially leafy greens, deteriorate after a few hours storage and should be used as soon as possible after treatment. It is concluded that the use of acetic acid on frese vegetables would provide considerable, but not necessarily complete, protection against *E. histolytica* cysts present on the foods.

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FURTHER OBSERVATIONS ON *TRYPANOSOMA ARIARII* OF COLOMBIA, SOUTH AMERICA

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In 1950, Groot, Renjifo and Uribe (1951) proposed the name *Trypanosoma ariarii* for a protozoön found in man in Colombia, South America. *T. ariarii* seems to be related to the human trypanosomes considered as *T. rangeli*. We retain, however, the specific name proposed by Groot *et al.* since the present data regarding the so called *T. rangeli* from human beings are not sufficient to permit a complete comparison with *T. ariarii*.

Later, Groot and Uribe (1951) demonstrated that *Rhodnius prolixus* was found infected in nature with *T. ariarii* and that the flagellates were present in the contents of the head as well as in the intestinal tract of the reduviid. Based on these observations, a systematic study was begun of the localization of *T. ariaii* within the infected *R. prolixus*. At the same time we were very much impressed with similar findings by several investigators who have worked with various American trypanosomes. C. Chagas (quoted by Wenyon 1926) described for the first time in 1909 flagellates of the trypanosome type (*T. cruzi*) in the body cavity fluid of *Triatoma megista* and stated that he had also seen them in the smears of the salivary glands. Brumpt was unable to demonstrate this phase, even when he examined reduviids with a heavy intestinal infection. Torres, in 1915 (quoted by Wenyon) failed to demonstrate flagellates in the body cavity fluid of *T. megista*, though he succeeded in infecting animals by allowing the bugs to bite through gauze, which prevented fecal contamination of the skin. Rey (1941), working in Colombia, found a trypanosome classified as *T. rangeli* in the salivary glands and hemolymph of two specimens of *R. prolixus*, and noted the presence of flagellates apparently in the protoplasm of cells. Later on, Muñoz (1945) found *T. cruzi* in the body cavity fluid of *R. prolixus*. Pifano and Mayer (1949) in Venezuela, found a flagellate classified as *T. rangeli* in the fluid obtained from the proboscis of 5 adult *R. prolixus*. Moreover, these authors reported the presence of a few flagellates in the salivary glands in one case. Romeo de Leon (1950), working in Guatemala studied *R. prolixus* heavily infected with a trypanosome considered as *T. rangeli*, but could not demonstrate the parasites in the salivary glands. Therefore, it appears that the presence of trypanosomes in the hemolymph or in the salivary glands of *Triatominae* is not a new finding, but that the observations are conflicting. However, in all these cases no information is given with regard to the duration of the infection in the bug, the temperature at which the reduviids were kept, the date of the infective meal, or the state of fasting, and whether the results were based on a single observation or on a series. As will be shown later, from our own experience, the lack of information on such points may explain some of the conflicting results.

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In the following pages are presented the preliminary results of our study of the behavior of *T. ariarii* in *R. prolixus*—mainly the invasion of the hemolymph and the salivary glands—both in experimental and natural infections, as well as the results of some experiments of transmission by biting. Although the number of reduviids involved in these studies and experiments is small, we believe that the results so far obtained may be of some value for a better understanding of the transmission of *T. ariarii* and of the life cycle of some of the American trypanosomes in the invertebrate host.

MATERIALS AND METHODS

Four series of trials are reported in this paper. In Observations Nos. 1 and 2, *R. prolixus* captured in nature (in a peasant hut where a human case of infection with *T. ariarii* had been found earlier) were used to investigate the possible presence of trypanosomes in feces, hemolymph and salivary glands. These insects were captured in Nilo, Colombia. The lot studied in Observation No. 1 was examined only once, within 48 hours after capture, while the *R. prolixus* studied in Observation No. 2 were examined over a period of three weeks or until the insects were sacrificed or died. During this time none of the insects were fed in the laboratory except those with proved invasion of the hemolymph, which were used later for transmission experiments. In Observation No. 3, experimentally infected *R. prolixus* were used. These insects—obtained by courtesy of Dr. E. Osorno—had been raised in the laboratory and were free from trypanosomes. They were infected by allowing them to feed on rats or dogs which had been infected with cultures of *T. ariarii*. All the reduviids used in these observations were kept at 23°C.

When flagellates were seen in fresh preparations of feces, hemolymph or glands, Giemsa-stained smears were made. In some cases paraffin sections of the glands and of the head of the insects were also made. In a few cases, the flagellates found in the invertebrate were inoculated into rats. In order not to kill the insects, the hemolymph was obtained by sectioning the distal portion of their limbs, and receiving in a cover glass the fluid which in some specimens flowed spontaneously, in others after a gentle pressure on the limb. In a similar way hemolymph could be obtained from the proboscis and from the antennae. The feces were obtained by exerting gentle pressure on the terminal segments of the abdomen and receiving in a cover glass the product expelled through the anus.

To study the salivary glands, a dissection of these was performed following the technique devised by Osorno, Uribe and Ramirez. In this technique, the head of the insect is gently pulled after sectioning the digestive tract, loosening the joints between the thorax and the head and performing a longitudinal incision in the middle of the sternal portion of the thorax (Ramirez 1951).

The experiments of transmission through the bite of *R. prolixus* (Observation No. 4) were performed in the following way: the reduviid was placed, with the head towards the opening, in a narrow test tube inside which it could not turn. The tube was lined with filter paper, and its mouth fastened to the rat with adhesive tape. In this way, fecal contamination of the skin could be prevented.

White rats, two to six days old, were used. The insects were allowed to feed for about 30 minutes.

The transmission was considered successful when *T. ariarii* could be demonstrated in the blood of the bitten rats either in films or in blood cultures taken within ten days following the bite of the reduviids. In order to ascertain the time which elapsed between the bite and first appearance of *T. ariarii* in the blood, some of the rats were examined every two hours during the 24 hours following the bite.

RESULTS

As will be shown below in detail, trypanosomes were found in the hemolymph and in the salivary glands of several *R. prolixus*. We observed that in no specimen could parasites be detected in the glands unless they were found in the hemolymph. The morphological aspects of *T. ariarii* in hemolymph, salivary glands and in paraffin sections of the head of these reduviids are in the course of study and will be published elsewhere.

At present, it is of interest to report that in the hemolymph the parasites are free in the fluid itself and in some cases we have found them within the protoplasm of cells. We do not know yet whether this is a case of simple phagocytosis. Different developmental stages of *T. ariarii* may be observed both in the hemolymph and in the salivary glands. At the beginning of the hemolymph parasitism, short crithidia and leptomonas-like forms seem to predominate and flagellates are scarce; later on, their number greatly increases and long crithidia and trypanosome forms are found. Sometimes, the number of parasites in the hemolymph is enormous, giving the fluid a cloudy aspect. In salivary glands obtained from insects after a short period of hemolymph parasitism the number of flagellates is small; however, when the parasites have been established in that fluid for a longer time, their number increases. In some cases, the glands were teeming with parasites and were practically converted into sacs of trypanosomes. When this was the case, the glands had a whitish appearance instead of the normal reddish color of glands free from parasites.

Observation No. 1.—Invasion of the hemolymph and the salivary glands of R. prolixus *captured in nature by a trypanosome having the morphological aspect of* T. ariarii.

The insects were sacrificed and studied within 48 hours following capture. Flagellates were found in the hemolymph in 9 out of 29 adults harboring trypanosomes in the intestinal tract. Of these 9, the salivary glands were examined in 6, and all were found to contain flagellates. The trypanosome invasion of the hemolymph and glands was observed in both males and females.

Observation No. 2.—T. ariarii *invasion of hemolymph and salivary glands of* R. prolixus *captured in nature, and periodically examined in the laboratory.*

Another lot of 29 adult *R. prolixus* infected with *T. ariarii* was studied, not only once but on repeated occasions for approximately three weeks. In the first

examination, which took place within the 24 hours following capture, flagellates were demonstrated in the hemolymph in 4 specimens (1 male, 3 females) and in 25 this fluid was found free from trypanosomes. After repeated study of the hemolymph, however, the parasites were finally found in 21 (9 males and 12 females) of the 25, reaching this fluid between the first and the ninth day after capture. In the remaining 4 (males) no parasites were ever observed in the hemolymph. It must be noted, however, that these four males died 2, 4, 8 and 11 days after capture, a period of time that in other specimens often proved to be too short for the invasion of the hemolymph. As can be seen in Observation No. 3, the flagellates may appear in the hemolymph as late as the 17th day of adult life.

Most of the 25 reduviids which showed flagellates in the hemolymph were used in transmission experiments as will be shown below and had their salivary glands examined. The glands were studied in 19 with the following results: no parasites were found in 3 (2 males, 1 female) examined on the first day, in 5 (3 males, 2 females) examined on the second day, in 1 female examined on the sixth day, and in 1 female examined on the eighth day after the hemolymph parasitism was established. Parasites were found in the glands in 1 male examined on the third day, in 2 (1 male, 1 female) examined on the sixth day, in 2 males examined on the ninth day, in 1 female examined after the fifth day, in 2 (1 male, 1 female) examined after the tenth day and in 1 female examined between the sixth and the eleventh day following the trypanosome invasion of the hemolymph.

We feel reasonably sure that the flagellates observed in the hemolymph and in the salivary glands of this lot of *R. prolixus* belonged to the species *T. ariarii*, since the parasites showed the morphological aspects of *T. ariarii* and since the bite of four of these insects produced a proved infection of *T. ariarii* in rats, as will be shown later.

Observation No. 3.—T. ariarii *invasion of hemolymph and salivary glands of* R. prolixus *infected in the laboratory.*

Five *R. prolixus* were infected in their first larval instar by allowing them to feed on rats infected with cultures of *T. ariarii*. The reduviids were infected approximately 5½ months before becoming adults. They were regularly fed on a normal guinea pig (free from trypanosomes) until they moulted for the fifth time. Thereafter, they were not fed, except those which were used in transmission experiments. In all these 5 adults, parasites were found in the hemolymph. In one male the parasites appeared in this fluid on the sixth day and in another male sometime between the eighth and seventeenth day of adult life. In the remaining three (females) it was not possible to determine on which day of adult life the parasites reached the hemolymph or whether the flagellates had already invaded this fluid during the previous instars.

Ten nymphs were infected through feeding on dogs or rats infected with cultures of *T. ariarii*. The insects moulted approximately one month later and

became adults (1 male and 9 females). Thereafter they were not fed except those used in transmission experiments. In all of them the parasites appeared also in the hemolymph between the fourth and the seventeenth day of adult life, approximately between 32 and 42 days after the infective meal.

It appears, therefore, that hemolymph parsitism with *T. ariarii* has been a constant feature in these 15 experimentally infected adult *R. prolixus* regardless of the instar in which they had their infective meal. However, the period of time necessary for the trypanosomes to reach the hemolymph does not appear to be uniform, even in specimens infected at the same time.

The salivary glands were studied in 11 out of these 15 specimens, with the following results: no parasites were found in one male examined on the first day and in one female examined on the third day after the hemolymph parasitism

TABLE 1

Relationship between the first finding of T. ariarii *in the hemolymph and the finding of the trypanosome in the salivary glands of adult* R. prolixus

	NUMBER OF DAYS SINCE FIRST FINDING OF T. ARIARII IN HEMOLYMPH								
	1	2	3	5	6	8	9	More than 10	Not determined with precision
Number of *R. prolixus* examined	4	5	2	2	3	2	2	6	4*
Number of specimens with salivary glands infected with *T. ariarii*	0	0	1	2	2	1	2	6	4
Number of specimens with salivary glands free from *T. ariarii*	4	5	1	0	1	1	0	0	0

* In one 6–11 days; in three, more than 5 days.

was established. Parasites were found in 2 (1 male, 1 female) examined on the fifth day, in 1 female examined on the eighth day, in 2 (1 male, 1 female) examined after the fifth day, and in 4 females examined after the tenth day following the trypanosome invasion of the hemolymph.

These results are similar to those mentioned in Observation No. 2: during the first days of hemolymph parasitism the salivary glands are free from *T. ariarii*, but later on the flagellates invade the glands. Table 1 summarizing the results discussed in Observations 2 and 3, shows this apparent relationship between the duration of the hemolymph parasitism and the invasion of the salivary glands.

Furthermore, two *R. prolixus* were infected in their first larval instar by allowing them to feed on a rat infected with cultures of *T. ariarii*; six months later, when they were in their nymphal stage, they were studied. These insects had fed for the last time, on a normal guinea pig, 57 days before examination. Both showed *T. ariarii* in their hemolymph. One was sacrificed and flagellates were found in the salivary glands. The other, as will be shown below, when allowed to bite a rat ten days after this first examination, transmitted *T. ariarii* to the rodent.

Observation No. 4.—Transmission of T. ariarii *to rats through the bite of naturally and experimentally infected* R. prolixus.

In view of the above observations, namely, that in no case when the hemolymph was free from parasites did the salivary glands show flagellates; that, once the parasitism of this fluid was established, some time had to elapse before *T. ariarii* could be found in the glands, and that the longer the hemolymph parasitism persisted, the heavier appeared to be the infection of the salivary glands, we were led to attempt to infect rats through the bite of *R. prolixus*.

TABLE 2

Results of biting of white rats by Rhodnius prolixus *harboring* Trypanosoma ariarii *in the hemolymph*

R. PROLIXUS NO.	SEX	TIME IN DAYS FROM INFECTIVE MEAL	TIME IN DAYS SINCE T. ARIARII WAS FIRST FOUND IN HEMOLYMPH	RESULTS OF BITING WHITE RATS
1[1]	male	165	1	Rat not infected with *T. ariarii*
2	female	unknown[3]	1	Rat not infected with *T. ariarii*
3	female	unknown[3]	2	Rat not infected with *T. ariarii*
4	male	unknown[3]	2	Rat not infected with *T. ariarii*
5	male	unknown[3]	5	Rat not infected with *T. ariarii*
4	male	unknown[3]	6	Rat not infected with *T. ariarii*
6[2]	female	48	17	Rat infected with *T. ariarii*
4	male	unknown[3]	19	Rat infected with *T. ariarii*
7[2]	female	68	27–29	Rat infected with *T. ariarii*
8[1]	nymph	167	more than 10	Rat infected with *T. ariarii*
9[1]	female	161	more than 10	Rat infected with *T. ariarii*
10	female	unknown[3]	more than 10	Rat infected with *T. ariarii*
11	female	unknown[3]	more than 13	Rat infected with *T. ariarii*
12	male	unknown[3]	more than 18	Rat infected with *T. ariarii*
8[1]	nymph	183	more than 26	Rat infected with *T. ariarii*
9[1]	female	184	more than 33	Rat infected with *T. ariarii*

[1] Infected in the laboratory in the first larval instar
[2] Infected in the laboratory in the nymphal instar
[3] Found infected in nature

Some of the *R. prolixus* studied in Observations 2 and 3 which showed *T. ariarii* in their hemolymph were used in these experiments.

We carried out 16 trials with 12 insects (11 adults and 1 nymph) and in each trial one insect was allowed to bite a young white rat. Nine insects were used only once, while two (Nos. 8 and 9) were each used in two trials and the remaining one (No. 4) in three trials. The results are given in Table 2 which shows that the biting of the infected *R. prolixus* did not produce infection in the rats before the 7th day or proved trypanosome invasion of the hemolymph. However, when hemolymph parasitism had been established for more than ten days, transmission through biting was always successful. Of the 8 specimens which transmitted *T. ariarii* through the bite, one was an experimentally infected nymph, three were experimentally infected adults (females), and four (2 males, 2 females) had been found infected in nature.

The time which elapsed between the bite and the first appearance of *T. ariarii* in the blood of six of the rats was 10 hours in two, 16 hours in one, 20 hours in one, 24 hours in one and 48 hours in one.

DISCUSSION

The results of Observation No. 4 support the view that some time must elapse between the finding of *T. ariarii* in the hemolymph and the ability of the infected adult *R. prolixus* to transmit the infection through the bite. This is clearly observed in the specimen (No. 4) which did not transmit the flagellate on the 2nd and on the 6th day of the hemolymph parasitism, but was able to transmit the infection on the 19th day. These results are in accordance with those of Observations 2 and 3 in which it was demonstrated that a certain time is necessary for the trypanosomes to reach the salivary glands through the hemolymph. All these results lead us to believe that the transmission through the bite is due in fact to the presence of trypanosomes in the saliva of the insects which is injected at the time of biting. Our limited observations seem to point to a cyclical development of *T. ariarii* in *R. prolixus*, in which the flagellates are first present in the intestine after the infective meal of the reduviid on a vertebrate with enough parasites in the peripheral blood. Later some of the flagellates migrate to the hemolymph and from this to the salivary glands. From the glands the trypanosomes can be transmitted—through the bite— into new vertebrates. As to the way in which the parasites pass from the intestinal tract into the hemolymph, we do not have at present any information.

The fact that two *R. prolixus* (Nos. 8 and 9) were able to infect two different rats in succession by biting seems to indicate that the bite continues to be infective once the salivary glands have been invaded by *T. ariarii*. Furthermore, our results show that transmission through the bite may be accomplished by males, females, or nymphs.

We have demonstrated that the presence of *T. ariarii* in the hemolymph and later in the salivary glands is, under the conditions of our observations, a rather constant feature. The possibility that these facts are due to the artificial conditions under which the reduviids were kept in the laboratory, to the state of fasting in some of them or to the traumatism involved in the repeated examinations to which the insects were submitted, seems unlikely since in some engorged *R. prolixus* found infected in nature, in which examination took place within 8 hours after capture, the parasites were already present in the hemolymph and in the salivary glands. Furthermore, although the number of specimens used was very small, we have regularly obtained infections of rats through the bite of *R. prolixus* in which hemolymph parasitism had been established for more than 10 days. From the evidence presented in this paper it is clear that transmission through the bite can be obtained in the laboratory and that this may be the case under natural conditions. However, the possibility of infection through contamination with the feces cannot be excluded since Groot and Uribe (loc. cit.), found in the feces trypanosome forms capable of infecting dogs.

SUMMARY

The presence of *T. ariarii* in the hemolymph and in the salivary glands of *R. prolixus* (both naturally and experimentally infected) is a rather constant feature. Furthermore the ability of *R. prolixus* to transmit *T. ariarii* through the bite has been proved. These data seem to point to a cyclical development of *T. ariarii* in *R. prolixus*, in which the flagellates are first present in the intestine after the infective meal. Later on, some of the parasites migrate to the hemolymph and from this to the salivary glands. From the glands the trypanosomes can be transmitted—through the bite—into new vertebrates.

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DIASONE, AVLOSULFON AND SULPHETRONE AS THERAPEUTIC AGENTS IN EXPERIMENTAL TOXOPLASMOSIS*

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This is a continuation of an earlier report on the effectiveness of Diasone and of Promin in the treatment of experimental toxoplasmosis in the white mouse (Cross, 1951). Since the present drugs of choice for human therapy, sulfapyridine and sulfathiazole (Mouriquand *et al.*, 1950), presumably fail to eliminate the carrier state in mice (Weinman, 1944; Sabin *et al.*, 1942), it was noteworthy that repeated trials of over a year's duration of Diasone and of Promin indicated an encouraging number of such eliminations. The difficulty of eliminating the carrier condition has also been emphasized recently by Ruchman's report (1951) of the recovery as long as 2 years after infecton of virulent *Toxoplasma* from brains of symptom-free rats although other body tissues were non-infective.

Numerous antibiotics, sulfas, arsenicals and essential nutrients have proved ineffective protection against death in experiments by us and by others (Sabin *et al.*, 1942; Sabin, 1942; Augustine *et al.*, 1944; Summers, 1949; Cross and Anigstein, 1948). The favorable results with sulfadiazine reported by Biocca and Nobrega (1945) could not be confirmed by us whether the drug was injected as recommended by them or fed continuously as 1% of the diet. Our success with Diasone, which they found ineffective, may be attributable to a difference in administration although their failure to report drug controls raises the question of accidental toxic dosage as a cause of fatalities.

A severe challenge has been preferred for these studies. Previous publications (Cross, 1951; Cross and Anigstein, 1948) outline the routine inoculation after which, during 5 years of experimentation, there have been no survivors of untreated, infected mice. Death usually occurs in 7 to 8 days. The two groups of untreated, infected mice listed in Table 1 illustrate this uniform fatality. Since the results with Diasone surpassed those with Promin when therapy was delayed by $\frac{1}{3}$ the survival period of the untreated mice, Diasone[2] (courtesy of Abbott Laboratories) was used as a standard for therapeutic evaluation of 2 other sulfones, Sulphetrone[3] (courtesy of Burroughs Wellcome and Co.) and Avlosulfon[4] (courtesy of Imperial Chemical Industries). Trials with Avlosulfon (4:4′: diamino diphenyl sulfone) have been reported by Summers (1949) as partially effective. Possibly our greater protection with this drug is also attributable to a difference in administration.

All of the drugs were given orally in the diet. Intermittent therapy followed

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[2] Disodium formaldehyde sulfoxylate diamino diphenyl sulfone.

[3] Tetrasodium 4:4-bis (γ-phenylpropylamino-diphenylsulfone-a:γ:a′:γ′-tetrasulphonate.

[4] 4:4′diamino diphenyl sulfone.

continuous feeding, not for the purpose of lessening the toxic reaction as in leprosy in man (Johansen *et al.*, 1949), but to counteract any possibility of the drug's discouraging the emergence of parasites that might have been sheltered from the drug's action by intracellular localization. Continuous Diasone feeding of 2 groups of mice for 4 and 6 weeks, respectively, preceded an omission of the Diasone: its renewal, omission and renewal (1 week each). Observable differ-

TABLE 1

Therapy

Comparison of Diasone, Avlosulfon and Sulphetrone

NO. OF MICE	DAILY INTAKE (MGS.)/MOUSE				DAYS SURVIVED
	Diasone 0.5%	Avlosulfon 0.1%	Sulphetrone 1.5%	Sulphetrone 3.0%	
5 Inf	0	0	0	0	7½(2 mice); 8(3)
8 Inf	0	0	36 1100/K	0	7(2); 8(1); 9(1); 11(1); 12(1); 14(1); 24(1)
3 Drug Controls, ExC—1600/K, Sulphetrone					
16 Inf	See Table 2	0	0	0	(11 donors) ExC
Additional Trial					Terminated, 45 days
5 Inf	0	0	0		7(2 mice) 7½(3)
4 Inf	0	0	0	141:4 wks 2500/K	10(1); 34(1); 43(1); 45(1,S?)
3 Drug Controls, ExC—5500/K, Sulphetrone					
4 Inf	17:4 wks 800/K	0	0	0	45(1) 45(3, ExC)
2 Drug Controls, ExC—1000/K, Diasone					
8 Inf	0	3½:4 wks 140/K	0	0	25(1) 45(1,S?) 45(6, ExC)
4 Drug Controls, ExC—170/K, Avlosulfon					

Therapy: Beginning 2½ days post inoculation, drugs given continuously 4 weeks, omitted and renewed 1 week each.

Inoculation: Subcutaneous, 0.4 ml. of emulsion of brain and Tyrode's solution.

Inf: Infected. ExC: In excellent condition. S: With symptoms.

ences in protection in the 2 groups were negligible and the shorter period was chosen for later trials. Space limitations prohibited using larger numbers of animals.

Table 1 shows that Diasone (0.5%) continued to suppress symptoms and protect against death. Sulphetrone gave partial protection with 3.0% but almost none with 1.5%. The effectiveness of a very small amount of Avlosulfon is of particular interest. Results with Avlosulfon surpassed those with Sulphetrone with $\frac{1}{30}$ the dosage and approximated those with Diasone with $\frac{1}{5}$ the dosage.

Francis & Spinks (1950) have listed a similar scale of results in trials against *Str. pyrogenes* and explain the varying effectiveness by the varying proportions of 4:4′: diamino diphenyl sulfone in the compounds. Unfortunately, the trial of Avlosulfon was necessarily terminated at 45 days post inoculation so that no investigation of the elimination of the carrier condition was possible.

Table 2 gives details of the administration of Diasone preceding the investigation for the carrier condition. Separate estimates of the daily intake of the drug in mgs/mouse were made for the continuous dosage period and for the following week-long feedings (Cross and Anigstein, 1948). The intake per kilo-

TABLE 2

Therapy

Preparation of Donors

NO MICE	DAILY INTAKE (MGS.)/MOUSE, 0.5% DIASONE IN DIET		DAYS SURVIVED	RESULTS
8 Infected	15 for 4 wks 23 for 1st 26 for 2nd 650/K 2nd	Plan 4:1:1	21(1 mouse); 22(1): 33(1): 58(4, donors) 64(1, donor)	5 donors, excellent condition.
8 Inf.	18 for 6 wks 25 for 1st 24 for 2nd 725/K 2nd	Plan 6:1:1	45(1, fighting?) 59(1); 76(3, donors) 82(3, donors)	6 donors, excellent condition.
3 Not Inf.	23 for 6 wks 26 for 1st 24 for 2nd 700/K 2nd	Plan 6:1:1	72 (All killed)	3 Drug controls excellent condition.
5 Inf.	None		7½(2 mice) 8(3)	All died in same 12 hours.

Therapy: Began 2½ days post inoculation (¼ survival period of untreated controls).
Inoculation: Subcutaneous. 0.4 ml. emulsion of brain and Tyrode's solution.
Plan 4:1:1; 4 weeks of continuous therapy; 1 week each, omitted, renewed, omitted and renewed. Plan 6:1:1: Substitute 6 for 4 in preceding scheme.

gram was based on the stationary growth and weight period of the final week. Probably the estimates are larger than the actual intake since it is impossible to prevent all food wastage.

Table 3 outlines the investigation for carriers in the symptom-free, Diasone-treated mice. The brain from each of the 11 donors was utilized for the intra-abdominal inoculations of 5 mice. The 11 groups of "initial recipients" totaled 55 mice. In 10 groups, after 27 to 31 symptomless days, pools of the longitudinal halves of the brains of 4 out of each 5 mice in a group were used for the intra-abdominal inoculation of 4 "final" recipients. In the 11th group in which several deaths occurred following atypical symptoms, 9 final recipients were used unsuccessfully in searching for definitive results. The 11 groups of final recipients

totaled 49 mice. After 28 symptom-free days (21 days in 1 group) all final recipients were killed.

Observations with the phase contrast microscope of preparations of peritoneal secretions from the 11 original donors and from dying recipients revealed no evidence of *Toxoplasma*. Such preparations from mice inoculated intra-abdominally with material known to be slightly infective routinely contain an abundance of parasites. Extremely high summer temperatures and consequent anorexia may have precipitated some deaths and, by reducing the commercial supply, forced the usage of smaller mice (5 gm. instead of 10 gm.). A unique abdominal

TABLE 3

Investigation for carrier condition in symptom-freed, diasone-treated mice

DONORS		RECIPIENTS		ELIMINATION OF CARRIER CONDITION
No.	Days PI	*Initial*: 5 per donor (1 group)	*Final*; 4 per pool (1 group)	
4	58	4 groups, All ExC, 31 days PI	4 groups, All ExC, 28 days PI	Indicated
1	64	1 group, 1 dead, 27 days*, 4 ExC, 31 days PI	1 group, 1 dead† 3 ExC, 28 days PI	Suggested
1	76	1 group Most survived. Atypical symptoms and death of several.	3 groups of 3 Most survived. Atypical symptoms and death of several.	Doubtful
2	76	2 groups, 1 dead† 1 missing, 8 ExC 27 days PI	2 groups, All ExC 28 days PI	Suggested
1	82	1 group, All ExC 28 days PI	1 group, All ExC 21 days PI	Indicated
2	82	2 groups, All ExC 28 days PI	2 groups, All ExC 28 days PI	Indicated

Inoculation: Intra-abdominal, 0.4 ml. (0.3 ml. in 5 gm. mice) of emulsion of brain and Tyrode's solution.

Pool: Longitudinal halves of 4 brains from each "Initial" group.

Days PI: Days post-inoculation when killed. ExC: In excellent condition.

* No parasites found in abdominal fluid (phase contrast microscope).

† As above plus evidence of trauma from overly large inoculum.

trauma was observed after death following obvious distress in one 5 gm. mouse during inoculation. Similar trauma had been recorded twice earlier. Only the 7 cases in which there were *no* deaths have been listed as "indicating" the elimination of the carrier condition although in the 3 additional cases listed as "suggested", it appears unlikely that death was caused by *Toxoplasma*.

SUMMARY

Diasone (0.5%) and Avlosulfon (0.1%), when administered in the diet, protect the majority of white mice from death in an otherwise rapidly fatal toxoplasmosis even when therapy is delayed by ⅓ of the survival time of the untreated animals. Sulphetrone used similarly at 1.5% is without effect and only slightly

protective when used at 3.0%. None of the drug controls showed any toxic reactions at these dosages. The carrier condition seems to have been eliminated certainly in 7 and probably in an additional 3 out of the 11 cases investigated. Since our results surpass those previously reported for Diasone and for Avlosulfon, it is possible that the method of administration (intermittent dosage following continuous dosage) contributes to the increased effectiveness.

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FIVE YEARS' OBSERVATIONS OF RURAL MALARIA IN EASTERN COLOMBIA

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The present paper summarizes the main features of the malaria studies carried out in Eastern Colombia from 1946 to 1950. The Villavicencio laboratory, built originally as a center for the study of jungle yellow fever in the region, was used as a base for our malaria studies. The laboratory was established twelve years ago, soon after the Bogotá-Villavicencio road was completed, the first and so far the only road built between the uplands of the interior and the low plains of Eastern Colombia. The laboratory was built, equipped and maintained jointly by the Rockefeller Foundation and the Colombian Government until 1948 when it became a dependency of the Colombian Ministry of Hygiene, supported entirely by Government funds.

The studies on jungle yellow fever were completed in 1946 and the laboratory activities shifted to malaria. The new studies followed two main lines: research on human malaria in the area and biological studies of the local anophelines. The first project was started by Dr. Rolla B. Hill of the Rockefeller Foundation, International Health Division; the second project was until 1948 under the direction of Dr. Marston Bates, also a member of the Rockefeller Foundation, International Health Division. To both of them the authors wish to express their gratitude for their personal help and encouragement and for making possible the development of malaria studies in Eastern Colombia.

In 1948, when the laboratory became directly attached to the Colombian Ministry of Hygiene and when the name of Instituto "Roberto Franco" was given to it, a program of malaria control was started. Since then research and control of malaria have been the main concern of the laboratory but not its only activity. Control and vaccination against yellow fever have also been a responsibility of the laboratory. Studies on human trypanosomes have been carried out in the last years and advantage was taken of the situation of the laboratory to make it a center for the study of the Macarena range. The Macarena mountains, situated 120 km. south of Villavicencio, are an uninhabited area, until recently practically unexplored, with a very rich flora and fauna; the area is now protected by law and made a National Reserve for natural history studies.

THE LLANOS OF COLOMBIA

Observation of a map of Eastern Colombia will show that, with the exception of a few minor elevations, all the territory lying east of the Andes is a vast plain of less than 500 m. altitude. The term Llanos, however, is not applied to the unbroken Amazonian forest but only, in local usage, to the savanna country of the Orinoco system. The Colombian Llanos, shown in Fig. 1, are bounded by the slopes of the Eastern Andes, the borders of Venezuela and Brazil, and their ap-

proximate southern limit is the course of the Guaviare river and the Macarena range. The observations reported in this paper are confined to this area.

The general features of the region have been discussed by Bates (1948) and by de Zulueta (1950a). It is sufficient to say here that most of the Llanos or plains are grass covered and that forest areas are only to be found along the rivers and around water collections. The clear-cut difference between savanna and forest is due mainly to soil formation and to some extent to the yearly burning of the savannas, a practice intended to produce fresh pasture for the cattle.

The savanna country begins usually 20 or 30 km. from the mountain front; this leaves a transition area between the Andes and the Llanos proper with a peculiar vegetation, differing from the forest galleries and the palm groves typical

TABLE 1

Meteorological data from the Villavicencio laboratory; average results from January, 1941 through December, 1950

MONTH	RAINFALL	DAYS WITH RAIN	MEAN TEMPERATURE	RELATIVE HUMIDITY		EVAPORATION OF WATER
				8 A.M.	12 M.	
	mm.		°C.	*per cent*	*per cent*	*mm.*
January	70.6	6.9	26.8	73.6	62.4	126.3
February	65.7	7.7	27.7	70.3	60.9	140.3
March	199.3	10.2	27.7	73.3	64.9	148.1
April	565.6	23.4	26.5	82.3	73.0	76.7
May	628.8	25.4	25.8	86.3	77.0	58.1
June	467.9	23.8	25.4	85.5	77.7	53.1
July	487.7	25.6	25.0	85.5	76.6	54.1
August	448.9	22.8	25.5	83.6	75.0	67.9
September	429.9	19.0	26.3	80.3	71.9	84.9
October	472.3	20.4	26.5	81.6	73.2	84.1
November	408.7	19.8	26.5	79.8	72.8	87.6
December	169.4	11.2	26.4	77.9	68.5	102.0
Average	4,414.8	216.2	26.3	80.0	71.2	1,083.2

of the Llanos. This piedmont area has considerable importance from the malaria point of view as will be shown later.

The main climatic feature of the Llanos is the alternation of a rainy season lasting from April to November and a dry season covering the remaining months. In Table 1 are summarized the meteorological records obained at the laboratory station in Villavicencio from 1941 to 1950. As can be seen, there is a heavy rainfall of nearly 4½ m. (175 inches) per year with a correspondingly high relative humidity and a very even temperature throughout the year. It can also be noticed that "dry" season months have a substantial amount of rainfall, although April and May, the beginning of the rainy season, have the maximum rainfall. The ten year records show that the peak of precipitation occurs within these two months, with the exception of two years in which the maximum rainfall occurred in June.

Villavicencio is situated at the very foot of the Andes and, as Bates (1948) pointed out, its rainfall is much influenced by the mountain front. There is less precipitation away from the mountains but records from various places in the Llanos, including Arauca, far away from the Andes, show that the annual rainfall in the Llanos is always above 1,500 mm. (ca. 60 inches).

The Llanos support a very scanty population devoted mainly to cattle breeding. Only at the foot of the Andes, in the vicinity of Villavicencio, there has developed an important agricultural population. The total population of the Llanos according to the census of 1938, the last available for this area, is only 107,327 inhabitants. This comprises the administrative divisions of Arauca, Casanare, Meta, Vaupés, Vichada and the areas of the departments of Boyacá and Cundinamarca lying east of the Andes. This is a strikingly low density considering that the area mentioned has a surface of 421,326 sq. km. There is no doubt that the population has increased since 1938 and that in all probability remote communities and Indian tribes were not then counted. From the data obtained by our laboratory in various places, we believe that the total population of the Llanos is roughly 150,000 inhabitants. If the census of 1938 is compared with previous records from the region, such as the census of 1905, it can be observed that the increase of population in the Llanos has been very small in recent years with the exception of the administrative division of Meta, where Villavicencio is situated, in which the population increased from 6,344 inhabitants in 1905 to 51,674 in 1938.

The population of the Llanos is mainly rural in character. Most of the inhabitants live in isolated cattle ranches built on the savannas or in huts made in forest clearings. The typical *llanero* dwellings have mud walls, whitewashed, with thatched roofs of palm leaves. In forest areas wooden planks and bamboo are used for walls and partitions. Usually clearings of grass and vegetation a few metres in radius are to be found round the *llanero* ranches. In some places the huts are of a more primitive type, consisting of little more than four poles supporting a thatched roof, a clear disadvantage for malaria control by DDT spraying.

THE ANOPHELINES OF THE REGION

Twenty-eight anopheline species have so far been found in Eastern Colombia. The distribution and behavior of *A. darlingi*, the most important from the malaria point of view, will be discussed under a separate heading. The typical savanna representatives are *A. pessoai*, *A. peryassui* and *A. parvus*. Their behavior in nature has been discussed in a separate publication (de Zulueta, 1950a). *A. albitarsis* is another anopheline commonly found in the Llanos. *A. argyritarisis* is usually found in the piedmont area but is not uncommon in the savanna country. There are records of *A. oswaldoi*, *A. punctimacula* and *A. mediopunctatus* from various places in the Llanos. *A. mattogrossensis* has seldom been found in the region. *A. kompi* and *A. thomasi* are present in some forest galleries of the Llanos. In the vicinity of Villavicencio, in the piedmont area, several species have been found which seem to be confined to this area. Among them the most abundant in nature is *A. rangeli* and of less common occurrence are *A.*

triannulatus, *A. davisi*, *A. strodei*, *A. benarrochi* and *A. pseudopunctipennis*. Of rare occurrence here are *A. eiseni*, *A. neomaculipalpus*, *A. gilesi*, *A. nimbus*, *A. anoplus*, *A. bambusicolus*, *A. boliviensis*, *A. homunculus* and *Chagasia bonneae* and *C. fajardoi*. The rarest of all the anopheline species is *A.* (*Lophopodomyia*) *squamifemur* of which there is a single record by Antunes (1937).

Of the 28 species mentioned above, only a few come in contact with man and are of importance from a malaria point of view. The results of house captures made in the neighborhood of Villavicencio and in other places in the Llanos show that *darlingi* is the most important house haunting anopheline, though, as will be shown later, its distribution is limited to certain areas; *albitarsis* and *pessoai* are commonly caught in houses in the Llanos and form the bulk of the house captures in places where *darlingi* is absent; *peryassui* and *argyritarsis* are of less common occurrence in houses in the Llanos. Other anophelines are only occasionally found in human dwellings, as is the case with *rangeli*, though this species is the most abundant anopheline in breeding places at the foot of the Cordillera.

Observations made in 1947 and 1948 and reported by Bates and de Zulueta (1949) show that in spite of the rather uniform climatic conditions met with throughout the year, there is a clear annual cycle among the anophelines of this region. The observations were made in the vicinity of Villavicencio, close to the mountain front, and it was found that *rangeli* was most abundant during the rainy season while *strodei*, *triannulatus* and *pseudopunctipennis*, on the other hand, had their peaks of abundance during the months with low rainfall and *strodei* and *pseudopunctipennis* could not be found at all in the area studied during the rainy season. The study of the fluctuations of the larval and adult populations seemed to indicate that they were probably due to factors affecting the adult anophelines rather than their larval stages.

A study of the behavior in nature of the adult mosquitoes was undertaken during the following years and the results obtained have been the subject of several publications (de Zulueta, 1950a, 1950b and 1952). A new method was evolved to determine the mosquito density in the savannas and it was found that during the rainy season it ranged from 1 to 3 million mosquitoes per sq. km., of which about a tenth were anophelines. These observations were complemented by ordinary stable trap captures and house captures and by catches of flying mosquitoes by means of nets fixed to moving vehicles. It was found also that during the rainy season all the areas of savanna studied were within the effective flight range of the savanna mosquitoes and all the observations pointed to the fact that the grass of the savannas is the true habitat of the mosquitoes breeding there.

Anopheles darlingi, MALARIA VECTOR

The first epidemiological studies made in 1946 showed a relation between the distribution of *darlingi* and high malaria indexes. The dissection of mosquitoes captured in the vicinity of Villavicencio confirmed the importance of *darlingi* as malaria vector in the region (Renjifo, 1948). As can be seen in Table 2, sum-

marizing these results, 14 *darlingi* females were found infected (10 stomachs and 10 glands) among 794 captured in houses. These captures were made from September to December 1946, that is, at the end of the rains and at the beginning of the dry season. Among the 22 *darlingi* captured in houses in September, none were found infected. Of the 295 captured in October, 13 were infected. In November only 1 positive finding was made among 284 and in December there was no positive specimen among 193 *darlingi* females captured in houses. Though the period of observation is perhaps too short, the results seem to indicate that the ability of *darlingi* as a vector decreases after the end of the rainy season.

Table 2 shows that no specimen from stable trap captures was found infected, but the sample is too small to justify any conclusions. If the results of the dissection of *pessoai* made elsewhere are taken into account, we believe that the fact of finding 177 females of this species not infected does not exclude this anopheline as a minor vector in the area studied.

TABLE 2

Results of dissection of stomachs and salivary glands of 1,048 anopheline females (Apiay-Pachaquiaro area, 1946)

ANOPHELINE SPECIES	HOUSE CAPTURES		STABLE TRAP CAPTURES	
	Mosquitoes dissected	Positive specimens	Mosquitoes dissected	Positive specimens
A. darlingi	794	14	7	0
A. pessoai	161	0	16	0
A. albitarsis	18	0	8	0
A. peryassui	9	0	1	0
A. strodei	3	0	1	0
A. punctimacula	—	0	1	0
A. rangeli	—	0	11	0
Unidentified	17	0	1	0
Total	1,002	14	46	0

The known distribution of *darlingi* in Eastern Colombia is given in Fig. 1. We must make clear that, in some of the localities within the *darlingi* area, this species is caught only in houses in the periphery of the villages and seldom ventures into an urban area. This is a common characteristic of all the anophelines found near the slopes of the Andes. In the Llanos proper, in areas free from *darlingi*, more anophelines are found in the houses of the villages and small settlements, but this may be due to the vicinity of breeding places and to the presence of shrubs and grass near the houses, rather than to a greater capacity for penetrating the urban areas.

A. darlingi is mainly found in the transition area between the Eastern Andes and the savanna country but it is found, nevertheless, in typical savanna country, away from the mountains. It is also present in some places along the narrow forest gallery of the river Meta. In the vicinity of Villavicencio, *darlingi* is found breeding at the edge of the forest in shaded parts of temporary water collections.

It is even found in road ditches sufficiently shaded. All these are man-made breeding places since all the open areas around Villavicencio have been made in recent years by clearing the forest; but in the savanna country we find *darlingi* in breeding places which can hardly have been altered by man. There, the typical breeding places of *darlingi* are permanent or semi-permanent lagoons, open to the savanna on one side and with forest on the other, or lagoons separated from the savanna by a thin ring of trees. These breeding places have such characteristic features, that they can easily be recognized from a plane or spotted in aerophotographs, and we have repeatedly confirmed the identification made from the air by larval

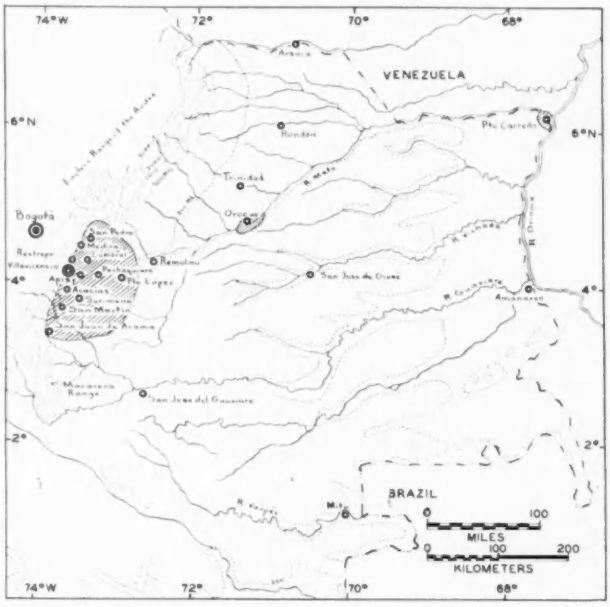

FIG. 1. Map of Eastern Colombia. Shaded areas show the known distribution of *A. darlingi*.

captures made on the ground. A typical example of these breeding places is given in Fig. 2. In such situations, *darlingi* is invariably found in the shaded parts, whereas other species such as *pessoai* and *peryassui* are found in the open side of the lagoons. This seems to indicate that the ovipositing females are influenced by physical factors such as shade and marginal vegetation rather than by the chemical composition of the water when laying their eggs, the chemical contents of the water being uniform throughout these lagoons. Furthermore, we have been unable to detect any significant difference in the chemical contents of samples taken from typical *darlingi* breeding places and from open lagoons, without marginal forest, where *darlingi* is never found. This is in accordance with our oviposition experiments carried out in the laboratory with *darlingi* (de Zulueta

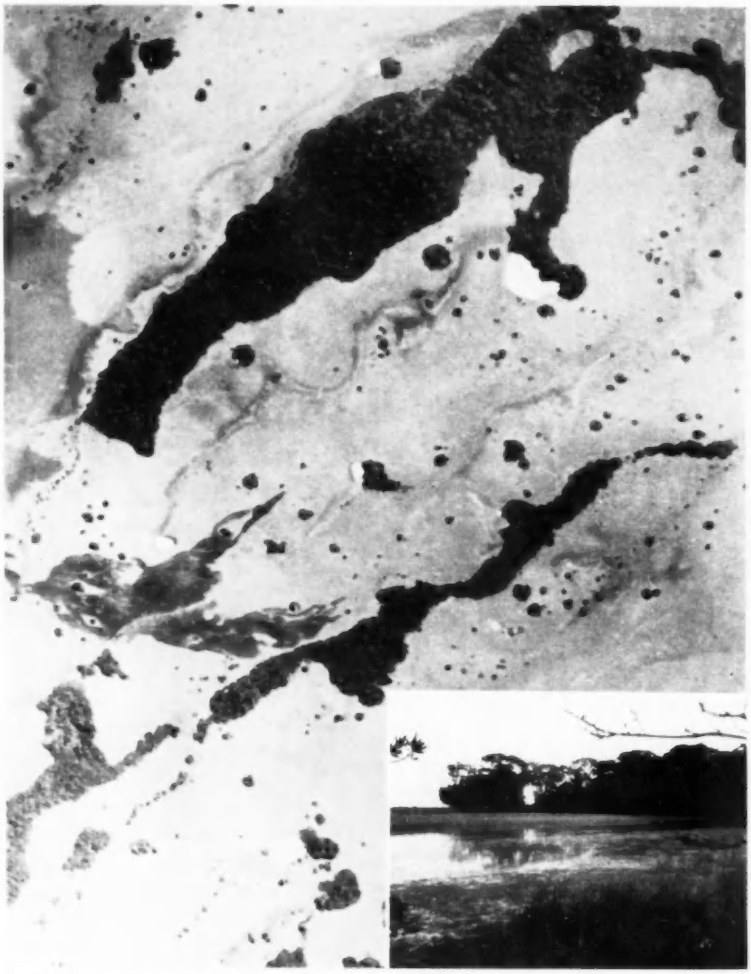

FIG. 2. Aerophotograph of savanna country in the Ariari region showing several lagoons partly screened by forest, typical breeding places of *A. darlingi*. The picture at the bottom corner, a ground photograph of one of these lagoons. Larvae of *A. darlingi* are found in the shaded parts under the trees. (Aerophotograph by courtesy of Compañia de Petróleos Shell de Colombia).

and Bates, 1948 and de Zulueta, 1950c). They showed that the oviposition behavior of *darlingi* is governed mainly by visual stimuli and to a certain extent by tactile and scent stimuli. The chemical contents of the water seem to have little effect on the oviposition of this species.

The population of *darlingi* is always small as judged by larval captures, even

in places where it is most abundant in houses. Using our methods for the measurement of adult mosquito density, we found (de Zulueta, 1952) that, in places where *darlingi* is the most common house haunting anopheline, the adult population of *darlingi* in the vicinity of the dwellings is nevertheless strikingly low.

Our field studies indicate that this species clearly prefers man to domestic animals when it can choose between them under identical conditions. This, added to its greater capacity for spotting a bait from a distance, contributes, no doubt, to make *darlingi* a more effective vector than other anophelines with a much higher density.

We have found repeatedly in the districts of San Juan de Arama and the Ariari that this species breeds at distances of 5 or more km. from human dwellings. This is particularly striking in the savannas of San Juan at the foot of the uninhabited forest of the Macarena, where *darlingi* is relatively abundant and where it is, no doubt, living without contact with man and is feeding on wild animals. We may add here that we have kept a colony of *darlingi* in the laboratory for nearly five years fed exclusively on peccaries.

Giglioli (1951) has raised the question whether *darlingi* can exist in nature entirely unassociated with man. He has found this species in forest areas of British Guiana where "its survival is conditional on feeding on wild animals", but he points out that even in those places its presence may be due to former migrations of man. In the case of the savannas of San Juan and the Ariari, the fact that *darlingi* is found in uninhabited areas cannot prove that this mosquito was not brought there by man. More studies are necessary to make certain that the species can live quite independantly of humans, but we believe it safer to assume that this is possible rather than to consider the association with man an essential condition in the life of *darlingi*.

We have drawn attention (de Zulueta, 1952) to the fact that this species was easily caught in daytime captures made in Venezuela and British Guiana (Gabaldón, 1949 and Giglioli, 1948), and that this never happened in our studies in Eastern Colombia, where we had to have recourse either to window traps, where the mosquitoes were collected early in the morning, or to captures made during the night at the time of biting. *Darlingi* enters houses only after sunset, usually late at night, but leaves them invariably before sunrise and this is characteristic of all the anophelines of the Colombian Llanos. It seems that *darlingi* is less closely associated with man in Eastern Colombia than in Venezuela and British Guiana and also that the natural environment is prefered to the interior of houses in Eastern Colombia but not in Venezuela and British Guiana where the mosquitoes rest indoors during the daytime. Soper (1951) raises the point that *darlingi* may be highly domestic in some places and in others semi-sylvatic; also that this species may be imported in some areas and indigenous in others. We believe that in the case of *darlingi* found in the savannas at the foot of the great forests of the Macarena and the Andes, we are dealing with a population which is firmly established, well adapted to its environment and which can live away from man and probably can survive when the link between man and mosquito has been severed by DDT house spraying.

MALARIA PREVALENCE

Malaria is endemic in all the Colombian Llanos, but the malaria indexes vary considerably according to the distribution of the anopheline species. Table 3 gives the results of the malaria surveys made before DDT was used. Population figures are based on our own data. The samples examined were taken from school children whenever possible and from among the general population when there were no schools. Spleens were palpated by the medical staff following Boyd's

TABLE 3

Malaria situation in Eastern Colombia prior to DDT

LOCALITY	DATE	POPULATION	POPULATION EXAMINED	ENLARGED SPLEEN				SPLEEN INDEX	PARASITE
				PDI&I	II	III	IV		
Villavicencio	March '46	11,109	228(1)	3	4	5	0	5	2
Acacias	March '46	2,306	308(1)	7	31	5	0	14	5
Restrepo	April '46	2,289	200(1)	9	7	1	0	9	3
Cumaral	April '46	854	93(1)	1	8	6	0	16	8
Puerto López*	April '46	628	100(2)	3	23	7	3	36	17
Pachaquiaro area*	April '46	250	49(2)	4	8	9	0	43	12
Apiay area*	July '46	491	245(2)	30	14	13	9	28	14
Orocué*	Aug. '46	800	76(1)	7	11	3	7	37	18
Trinidad	Aug. '46	900	80(1)	5	4	2	0	14	4
Surimena*	Sept. '46	300	34(2)	6	1	2	0	27	12
Concepción area*	Sept. '46	150	25(2)	5	2	0	1	32	4
Remolino	March '48	500	60(1)	7	0	0	0	12	5
San Martín	June '48	1,684	100(1)	—	—	—	—	—	10
Ariari area	June '48	600	183(2)	—	—	—	—	—	40
San José del Guaviare	Oct. '48	200	141(2)	—	—	—	—	—	0
Amanavén area	Nov. '48	100	28(2)	—	—	—	—	—	0
San Felipe area	Nov. '48	40	27(2)	—	—	—	—	—	0
Arauca	Dec. '48	1,754	150(2)	4	2	0	0	4	3
Medina	Dec. '48	711	100(2)	—	—	—	—	—	0
San Pedro & El Engaño	Dec. '48	800	127(2)	—	—	—	—	—	1
Medina area	Dec. '48	1,000	121(2)	36	16	10	0	51	20
Rondón	Jan. '49	345	91(2)	—	—	—	—	—	0
San José de Ocuné	April '49	300	93(2)	—	—	—	—	—	0
San Juan de Arama*	August '49	300	50(2)	17	5	1	0	46	18

* Localities haunted by *A. darlingi*.

(1) School children.

(2) General population.

method and, when unqualified assistants were making the surveys, only blood films were taken. Of the malaria parasites, *P. falciparum* is slightly more abundant in the Llanos than *P. vivax*. Among the 6,888 people examined, the distribution of the malaria parasites was as follows:

	P. vivax	*P. falciparum*	*P. malariae*
Number of positive cases	248	264	24
Per cent distribution	46	50	4

Malaria has a rural character throughout Eastern Colombia. As can be seen in Table 3, malaria indexes are always lower in the villages than in the neighboring rural areas, and in many positive cases found in the villages it was possible to trace the origin of the infection to the countryside. This is mainly due to the fact which we mention above, that the house haunting anophelines, including *darlingi*, seldom venture into urban areas.

Malaria indexes are usually higher in the areas where *darlingi* is present. Most of the savanna country is free from *darlingi* and, though the savanna anophelines are extremely abundant there throughout the nine months of the rainy season, the malaria indexes are nevertheless remarkably low. The presence of *darlingi* is probably not the only reason to account for the high malaria prevalence found at the foot of the Cordillera. The Ariari valley, the Medina area and in general all the stretch of country near the slopes of the Andes have a rich soil which yearly attracts a considerable number of immigrants from other parts of the country free from malaria, and the presence of people with no immunity probably has some effect on the malaria indexes. The poor soil of the savannas, on the other hand, is hardly fit for agriculture; there a more stable population is devoted mainly to cattle breeding and the lack of newcomers has probably some bearing on the low malaria indexes.

Seasonal fluctuations of malaria are less marked than could be expected from considerable variation in rainfall through the year; in places where malaria surveys were carried out during the dry season and during the rainy season as well, there was only a slight difference in the results. Malaria transmission is probably higher during the wet months but we have direct evidence that people contract the disease during the dry season. We know from Villavicencio records, covering several years, that *darlingi*, though more abundant during the rainy season, is to be found all through the year. Besides, if 60 per cent relative humidity (8 A.M. readings) should be taken as the level above which malaria transmission can occur (Russell *et al.*, 1946, p. 360), our metereological records show that at least the Villavicencio region is well above that level throughout the year.

During the time of our studies, we have seen a spontaneous reduction of malaria without the use of DDT. Some of the places where this was observed are so far away from the sprayed areas that we can be sure that the reduction was not influenced by the malaria control campaign. Seasonal fluctuations can also be excluded since the malaria surveys were carried out at the same time of year. The following findings well illustrate the spontaneous decrease of malaria in the Llanos:

	SPLEEN INDEX		PARASITE INDEX	
	1946	1949	1946	1949
Trinidad	14	3	4	3
Orocué	37	10	18	7
Surimena	27	9	12	5
La Concepción (Acacias)	32	12	5	4
Guacavía (Restrepo)	22	11	0	0

Gabaldón (1949) has called attention to the cyclical periodicity of malaria in Venezuela; the malaria peaks tend to occur there every five years and this cyclical trend coincides with our own observations in Eastern Colombia which seem to confirm the periodicity of malaria in the northern part of South America.

MALARIA CONTROL

Our malaria control program was based on DDT house spraying, treating not only the interior of the dwellings but also the corridor or entrance of the houses where the country people often sit and rest. The first trials with DDT took place in the Villavicencio area in 1948 but, owing to the limited means at our disposal, we could never make a complete malaria campaign, not being able to cover even all the *darlingi* area. Our malaria control program was complemented with DDT

TABLE 4

Effect of DDT in three rural districts in Eastern Colombia

LOCALITY	POPULATION	POPULATION EXAMINED	TIME OF DDT SPRAYINGS	TIME OF SURVEYS	SPLEEN INDEX	PARASITE INDEX
Apiay area	491	245	—	July '46	28	14
Apiay area	491	196	—	July '48	—	14
			Aug. Dec. '48			
Apiay area	491	150	Feb. Aug. '49	Aug. '49	4	0
Apiay area	491	150	Jan. June '50	Aug. '50	6	0
Puerto López	628	100	—	Apr. '46	36	17
Puerto López	628	100	—	July '46	53	25
Puerto López	628	68	May Nov. '48	Mar. '49	7	2
			July '49			
Puerto López	628	61	July '50	July '50	2	0
Pachaquiaro area	250	49	—	Apr. '46	43	12
Pachaquiaro area	250	35	—	Jul. '46	54	14
Pachaquiaro area	250	45	Jul. Nov. '48	Mar. '49	13	2
Pachaquiaro area	250	40	July '49	Apr. '50	22	10
Pachaquiaro area	250	43	July '50	Aug. '50	9	2

fogging, in villages free from anophelines, by fog applicators operated from the ground. In 1950 the population protected by DDT in Eastern Colombia was 31,241 inhabitants.

Table 4 summarizes the effect of DDT spraying in three rural communities with high malaria transmission due to *A. darlingi*. In the Apiay area the time elapsing between sprayings was less than 6 months, in Puerto López and Pachaquiaro from 6 to 11 months. As can be seen, in Apiay and Puerto López the parasite index was reduced to 0, a thing which never happened spontaneously in places where a reduction of malaria took place without DDT. The malaria reduction was effected sooner in Apiay than in Puerto López. The results from Pachaquiaro seem to indicate that the rate of spraying was inadequate, though this settlement received the same treatment as the village of Puerto López, where we secured satisfactory results. This may be due to the fact that in Pachaquiaro the dwellings are much more scattered and more exposed to the anophelines than in Puerto

López. In the trials we are discussing, during 1948 and 1949, DDT (technical grade) was sprayed at the rate of 1 gm. per sq. m. of treated surface, and in 1950, 2 gm. of DDT (wettable powder) per sq. m. were used.

Biological assays were made to ascertain the residual effect of DDT under local conditions. We followed the technique recommended by Pampana (1948) using Petri dishes stuck to the treated walls with adhesive tape and leaving the insects in the Petri dishes for 30 minutes. We used *darlingi* females from our colony bred under uniform conditions. Various types of wall were studied; painted wood, unpainted cement and whitewashed adobe or dried mud. This was done on the inner walls of ordinary houses in Villavicencio. Control experiments

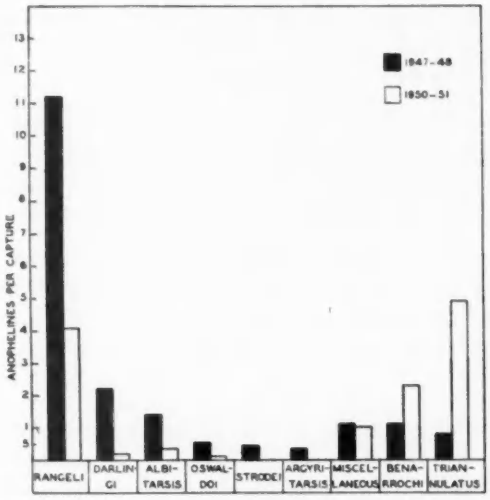

FIG. 3. Relative abundance of anophelines in stable trap captures made at El Chircal (Villavicencio). Based on the results of monthly captures made with animal bait from May 1947 through April 1948 and from May 1950 through April 1951.

using the same technique were performed on untreated walls in the laboratory. The treated walls had received 2 gm. of DDT per sq. m. either in the form of technical grade (Dupont) or as wettable powder (Pamisc, Pennsalt Corporation). As in the experiments of Maier *et al.* in Venezuela (1948), the residual effect of wettable powder lasted longer on these rough surfaces than that of the DDT technical grade. Up to the 5th month, all the *darlingi* females were killed by contact with the Pamisc treated walls; on the surfaces treated with DDT technical grade, this happened only up to the 2nd month.

Observations were made on the local anopheline populations before and after the use of DDT in the region. Fig. 3 is based on the results of stable trap captures made from 1947 to 1951 at El Chircal, a place situated near the Villavicencio laboratory, where experience showed that we could obtain a representative sample of

the local fauna. As can be seen, there was a considerable change in the species composition between 1947–48 and 1950–51; yet nothing was changed around the site of the stable trap, and climatic conditions, as judged by routine metereological observations, were unmodified. We must notice, however, that, in general, deforestation has increased in the Villavicencio region during the last years but we do not think that this alone could account for the shift in the species composition.

The obvious change in this area has been the introduction of DDT; all the houses in the neighbourhood of the trap were treated with DDT seven times from August 1948 to December 1950. The reduction of *darlingi* and its apparent eradication with DDT house spraying has been interpreted elsewhere as due to this insecticide. Here we have a marked reduction of the *darlingi* population but DDT cannot explain the reduction or disappearance of *rangeli*, *oswaldoi*, *strodei* and *argyritarsis*, since we know that these species were seldom caught in human dwellings in this area prior to the use of DDT. And it is more difficult to understand how DDT could account for the increase of the *triannulatus* and *benarrochi* populations. We think it likely that DDT house spraying had some effect on the decrease of *darlingi* but the changes observed in the other species make it clear that other factors of a more general nature must have been at work.

SUMMARY

Malaria is endemic in all the region between the slopes of the eastern range of the Andes and the borders with Venezuela and Brazil. The malaria indexes vary according to the distribution of the anopheline species; of the 28 anophelines found in the area, *A. darlingi* has proved to be, as in other parts of South America, the most effective vector; its distribution, however, is restricted to certain areas. Most of the savanna country of Eastern Colombia is free from *darlingi* and, though the savanna anophelines such as *A. pessoai*, *A. peryassui* and *A. parvus* are extremely abundant throughout the nine months of the rainy season, there are always low malaria indexes.

The fertility of the soil in the areas covered by *darlingi* has attracted in recent years a considerable number of agricultural laborers from other parts of the country, but the poor soil of the savannas is hardly fit for agriculture and there a more stable population has been devoted mainly to cattle breeding. Malaria has a rural character throughout Eastern Colombia. The house-haunting anophelines, including *darlingi*, seldom venture into the urban areas; they are not found resting in houses during the daytime, thus greatly reducing the effect of DDT. *A. darlingi*, though more closely associated with man than the other anophelines, is nevertheless found also in uninhabited areas and feeds normally on wild animals.

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THE INFECTION OF MOSQUITOES BY *PLASMODIUM VIVAX* (CHESSON STRAIN) DURING THE EARLY PRIMARY PARASITEMIAS

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Investigations reported by Eyles, Young and Burgess (1948) demonstrated the infection of mosquitoes by *Plasmodium vivax* during asymptomatic parasitemias. Although they found infections significantly lower in asymptomatic cases than in symptomatic, their results indicated that *vivax* malaria patients are potentially infective whenever parasites are present. These investigations were limited to certain asymptomatic parasitemias other than those present in an early primary infection. The purpose of the present report is to describe findings during these early primary parasitemias.

METHODS

Neurosyphilitic patients were inoculated by the bites of infected mosquitoes with the Chesson strain of *P. vivax*. Shortly after parasites appeared in the peripheral blood daily feedings of laboratory reared *Anopheles quadrimaculatus* were initiated. At least 100 *A. quadrimaculatus* were fed in each lot, and an average of 34.5 per lot were dissected 10 ± 1 days later to determine the number infected and the average number of oocysts per infected gut. Daily feedings were continued as long as practicable or until it was necessary to interrupt the clinical attack with a suppressive drug.

An effort was made to record complete parasitological and clinical data during the course of feeding. Daily prefeeding blood smears of the Earle-Perez (1932) type were made and the total parasite densities recorded in terms of cubic millimeters of blood. Male and female gametocyte densities were normally determined by examination of thin smears and were recorded on the basis of white blood-cell counts. Usually the gametocytes found during search for 200 white blood-cells were used in determining the density. The gametocytes were then expressed in numbers per cmm. of blood by means of a factor determined by white cell counts made on the prefeeding Earle-Perez smear. In several cases, where no gametocytes were found by normal examination procedures, examination of as much as 0.1 cmm. of blood on the thin film was undertaken to determine the presence of gametocytes at low densities.

Oral temperatures were taken at four-hour intervals until the first elevation was detected, after which temperatures were taken hourly.

OBSERVATIONS

Complete mosquito feedings were done during the early days of parasitemia in 24 primary attacks of *P. vivax* (Chesson strain). The feedings were usually initi-

[1] The author wishes to express his appreciation to the staff of the Milledgeville State Hospital for the cooperation which made this study possible.

ated during the first three days of demonstrable parasites and were continued as long as practicable or until suppressive treatment intervened. Table 1 summarizes the observations on the 24 patients. Parasites appeared on the average about 12 days after inoculation and first demonstrable gametocytes, on an average, about six days later. First infected mosquitoes were obtained on an average about 16 days after inoculation of the patient, or on the fourth day of detected para-

TABLE 1

Characteristics of early parasitemias in 24 cases of P. vivax *(Chesson)*

PATIENT NO.	DAYS AFTER INOCULATION					
	First Parasite	First Gametocyte	First Infected Mosquitoes	First Fever 100+	First Fever 102+	First Fever 104+
G 284	9	18	15	10	11	—
G 406	10	16	15	15	15	18
G 278	10	17	16	15	15	17
G 20	10	18	16	13	15	—
G 411	11	16	16	13	13	14
G 276	11	18	15	13	15	—
G 190	11	17	16	13	17	17
G 78	11	18	17	11	12	14
G 312	12	18	16	13	14	17
G 171	12	17	15	13	13	15
G 319	12	17	15	15	15	—
G 349	12	17	15	15	15	—
G 282	12	17	15	13	13	15
G 401	12	18	16	15	18	19
G 82	12	19	15	14	15	19
G 249	12	19	18	12	13	14
G 302	12	17	16	13	15	15
G 66	13	17	15	13	13	17
G 412	13	19	16	15	15	17
G 326	13	19	16	17	17	18
G 330	13	19	18	15	15	16
G 404	13	20	17	15	15	17
G 86	14	20	17	16	18	21
G 297	14	20	17	17	17	19
Range	9–14	16–20	14–18	10–17	11 18	14–21
Average	11.8	18.0	16.0	13.9	14.8	16.8
Median	12.0	18.0	16.0	13.5	15.0	17.0
Mode	12.0	17.0	15.5	13.0	15.0	17.0

sitemia. In all cases but one (G 411) the mosquito infections appeared before gametocytes were demonstrable by routine blood examinations and averaged about two days earlier. In the exception, gametocytes were first demonstrated and mosquitoes were first infected on the same day. In four cases the first onset of fever (100°F. or more) appeared on the same day that mosquitoes were first infected, but this onset preceded first infections in one case by six days. The onset of fever occurred, on an average, two days before first infection of mosquitoes and two days following demonstrable parasites. Fever of 102°F. or more

was usually attained a day later, but the first fever of 104°F. or more appeared on an average three days following the first onset, or about one day following the first infections in mosquitoes.

In table 2 a summary of parasitological and infectivity data from 28 cases where serial feedings were done during the first ten days of parasites is expressed in terms of days of parasite patency. Table 3 illustrates the infection incidence

TABLE 2

Infectivity of Chesson P. vivax *during first 10 days of parasite patency (28 cases)*

DAY OF PARASITES	PARASITES PER CMM.		GAMETOCYTES PER CMM.		INFECTIVITY TO MOSQUITOES				
	Range	Average	Range	Average	No. of Lots Fed	No. of Lots Infected	No. of Mosquitoes Dissected	Per cent Infected	Average Oocysts Per Gut
1	20– 70	45	0	0	2	0	127	0.0	0.0
2	30– 230	78	0	0	5	0	242	0.0	0.0
3	30– 1,800	551	0	0	13	1	582	0.3	1.0
4	20– 7,272	1,683	0	0	23	10	842	9.4	2.3
5	380–18,360	4,608	0– 38	2	24	14	842	30.8	7.8
6	1,680–54,000	10,721	0–123	21	26	21	819	43.8	13.3
7	1,490–49,680	17,161	0–200	56	24	23	661	61.9	33.2
8	5,184–72,000	22,635	0–363	139	21	17	631	40.1	31.0
9	2,856–51,840	24,707	0–804	170	11	8	357	26.3	47.7
10	3,000–52,200	22,630	33–513	188	10	8	430	35.8	12.9

TABLE 3

Mosquito infections with Chesson P. vivax *during the first seven infective days (24 cases)*

IN-FECTIVE DAY	PARASITES PER CMM.		GAMETOCYTES PER CMM.		INFECTIVITY TO MOSQUITOES				
	Range	Average	Range	Aver-age	No. of Lots Fed	No. of Lots Infected	No. of Mosquitoes Dissected	Per cent Infected	Average Oocysts Per Gut
1	756–18,360	4,977	0– 52(1)	2	24	24	872	32.6	3.9
2	970–54,000	11,310	0– 90(8)	18	23	22	816	41.4	8.3
3	1,680–32,112	14,886	0–265(13)	71	21	19	594	61.1	17.5
4	6,000–72,000	24,295	0–804(16)	166	17	16	508	74.0	44.4
5	6,120–36,720	21,491	27–363(10)	188	10	9	312	45.8	16.2
6	2,856–43,200	21,576	34–360(9)	165	9	7	313	31.0	41.6
7	3,000–52,200	21,027	29–513(7)	185	7	6	247	25.1	24.3

( ) Figures in parenthesis are cases showing gametocytes.

and intensity in the lots of mosquitoes fed on 24 patients during the first seven infective days. It will be noted from tables 2 and 3 that the early mosquito infections were usually of a fairly low grade, both in regard to per cent of specimens infected in each lot and in numbers of oocysts found in the infected specimens. However, all patients infected some mosquitoes by the sixth day of demonstrable parasitemia at the latest.

In an effort to demonstrate low level gametocytemias in the peripheral blood

of patients infecting mosquitoes previous to normally detectable gametocyte levels, 0.1 cmm. of blood was examined during the first four days of infectivity as well as on the day previous to infectivity in ten patients. Table 4 summarizes the results of these examinations. No gametocytes were demonstrable by this method on the day previous to infectivity, but on the first day of infectivity four of ten cases had demonstrable gametocytes, although they were few in number (10 to 20 per cmm.). On the second infective day gametocytes could be demonstrated in all cases, ranging from 10 to 50 per cmm. of blood. The following day gametocytes could be demonstrated in all but three cases by normal procedures, and were present in these three when 0.1 cmm. of blood was examined.

TABLE 4

Gametocyte densities in 10 cases during first 4 infective days, thin blood films by normal examination (200 w.b.c.) and by examination of 0.1 cmm. of blood

PATIENT NO.	GAMETOCYTES PER CMM.									
	Normal examination (200 w.b.c.)					Examination of 0.1 cmm. of blood				
	Day*									
	−1	1	2	3	4	−1	1	2	3	4
G 412	0	0	0	0	36	0	20	10	20	—
G 401	0	0	0	31	235	0	10	10	—	—
G 349	0	0	0	37	84	0	10	10	—	—
G 406	0	0	27	74	73	0	0	—	—	—
G 282	0	0	0	120	168	0	0	20	—	—
G 171	0	0	0	49	192	0	0	10	—	—
G 404	0	0	0	0	49	0	10	10	20	—
G 20	0	0	0	265	148	0	0	30	—	—
G 312	0	0	0	162	215	0	0	50	—	—
G 284	0	0	0	0	177	0	0	10	10	—

* Day 1 is first infective day.

DISCUSSION

The results obtained in this study are probably of some epidemiological importance. Although the first onset of fever invariably accompanied or preceded the successful infection of mosquitoes, the first frank malarial paroxysm often did not appear until several days later. It would be unusual for the naturally acquired *vivax* malaria infection to be terminated or suppressed in indigenous populations of endemic areas before the infected individual became a potential source of mosquito infections. The processes of specific diagnosis or recognition of the illness as caused by a malaria infection and the initiation of specific treatment in the infected individual would probably delay his elimination as a source of mosquito infection for at least the first two or three infective days, and possibly much longer.

In addition to the period preceding antimalarial treatment, there may be a day or two following treatment during which the patient is still infective to

mosquitoes. In our experience the effect of the better suppressives on gametocyte viability and density is variable. In seven cases of *P. vivax* (Chesson strain) where mosquito feedings were done before and after treatment with chloroquine, infectivity persisted in six for one day, and in two of these cases for two days, following medication.

The infectivity of low gametocyte densities in *P. vivax* has been noted previously by several investigators. Boyd and Kitchen (1937) were able to infect some specimens of *A. quadrimaculatus* when *vivax* gametocyte densities were less than 10 per cmm. in terminal asymptomatic parasitemias but noted a higher minimal infection density at the beginning of an attack. Eyles, Young and Burgess (1948) were able to infect about one-fifth of *A. quadrimaculatus* lots when fed on patients with asymptomatic or symptomatic parasitemias when fewer than 10 male gametocytes per cmm. were present.

In the present study mosquito infections were attained, with one exception, at least one day before gametocyte levels had reached an average of about 42 per cmm. (by normal examination procedures), and infections were found on an average two days before this density was reached. However, when as much as 0.1 cmm. of blood was examined in ten cases to determine presence of gametocytes, such forms were found invariably on the day after first infectivity, and in four of ten cases on the first infective day. It is evident that in many cases gametocyte densities of less than 10 per cmm. are able to produce some infections in mosquitoes.

It is noteworthy that in mosquito feedings done during the first few days of parasite patency in six symptomatic relapses of this strain of *P. vivax*, infections were produced invariably before appearance of demonstrable gametocytes and on the same day or one to two days in advance of the appearance of the first onset of fever. Often these mosquito infections were of a good quality, much better than those usually obtained from comparable primary parasitemias. These results are probably similar to those of Eyles, *et al.* (1948) in feedings done on patients with preclinical asymptomatic parasitemias in relapsing cases.

SUMMARY AND CONCLUSIONS

Complete mosquito feedings were done on 24 patients with early primary parasitemias of *Plasmodium vivax* (Chesson strain). The average infection produced infected mosquitoes on the fourth day of demonstrable parasites, two days before gametocytes could be discovered by normal examination of blood smears, two days following the onset of fever (100°F. or above), and about one day previous to the first fever of 104°F. or more.

It is concluded that these early parasitemias may be of epidemiological importance, in that, even in endemic areas where good therapeutic measures are available, the primary attack would provide a reservoir for mosquito infection before it could be terminated or suppressed. In indigenous populations of endemic areas it would be unusual for the naturally acquired *vivax* infection to be terminated or suppressed before the infected individual became a potential source of mosquito infections.

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THE EPIDEMIOLOGY AND CONTROL OF ORIENTAL SORE IN ABRUZZO, ITALY[1]

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The experimental control project described in this paper was carried out during the years 1948 to 1950 in Abruzzo, Italy. It was intended to determine whether the transmission of oriental sore could be prevented solely by the application of residual DDT to the interior of houses and stables.

For this purpose there was chosen a district located between the two rivers Tordino and Vomano, about 10 kilometers wide and extending 20 kilometers inland from the Adriatic Sea. This district covers the entire territory of the following four towns of the Province of Teramo: Roseto degli Abruzzi, Morro d'Oro, Notaresco and Castellalto. (See map.) This area lies in the center of a region in which oriental sore has been known to be endemic. The presence of cutaneous leishmaniasis in the Adriatic region was first reported by Monacelli (1934). He and other workers showed that cases of the disease could be observed in a large zone along the Adriatic, covering the Abruzzo and Marche regions.

Most of the early studies on leishmaniasis in this region were made by dermatologists or physicians and were more concerned with peculiar clinical and pathological forms than with giving an idea of the real incidence of the disease. On the other hand, the reports of public health officers have always indicated that the incidence is rather low. According to Bevere and Tobia (1947) a total of only 115 cases of leishmaniasis had been officially reported in Abruzzo and Marche together during the eighteen years from 1927 to 1945.

EPIDEMIOLOGICAL INVESTIGATIONS

Three different species of *Phlebotomus* are present in the region: *P. papatasi*, *P. perniciosus*, and *P. perfiliewi*. The last is the most abundant and has been considered to be the vector.

Our study started with an epidemiological investigation to determine the real incidence of oriental sore in the area to be treated with DDT (population two-thirds rural, one-third urban), and in two entirely rural test zones, portions of Giulianova and Atri, located on either side of the experimental zone, as shown in the map. This investigation was carried out by the examination of the entire

[1] This work was carried out with funds given by the Italian High Commissariat of Health (ACIS). Dr. Giovanni Rendina of the ACIS was a very active collaborator in both the epidemiological investigations and control work. Facilities and collaboration were also provided by Dr. Amalfitano and Dr. Di Meglio, Provincial Health Officers of Teramo, Dr. Prosperi, Director of the Health Laboratory in Teramo, and Mr. Tamburi of the Health Office in Teramo.

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population in their own houses, in the towns and villages as well as on the farms. For each individual it was determined whether or not he showed lesions or scars of oriental sore. The doubtful lesions were examined microscopically.

A total of 28,599 persons were examined by this method, with the following results:

In the centers of Roseto (including the smaller centers of Montepagano, Cologna, Cologna Spiaggia), Morro d'Oro, Notaresco, and Castellalto (including the smaller center of Castelbasso), 9,410 persons were examined. Of these, 191 (2 per cent) were found with active lesions of cutaneous leishmaniasis, 1,327 (14.1 per cent) showed the scars, and 7,892 were negative.

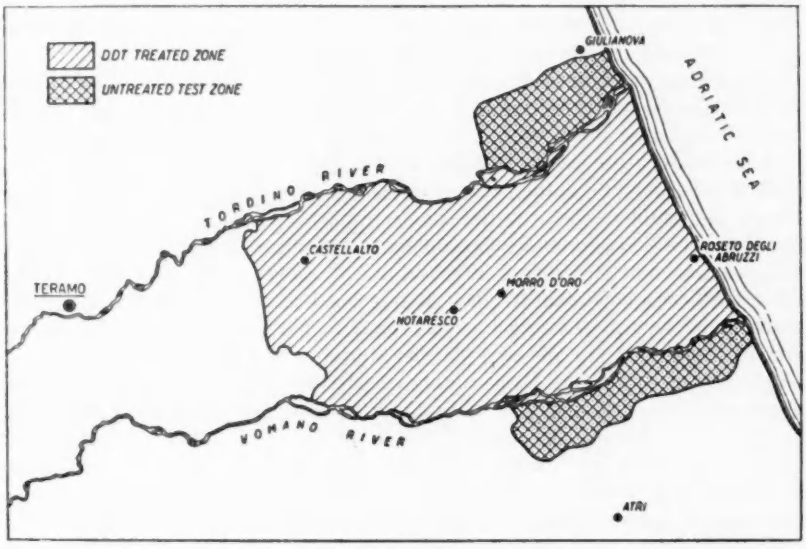

FIG. 1

On the farms of Roseto, Morro d'Oro, Notaresco, Castellalto, Giulianova and Atri, a total of 19,189 persons were examined. Of these, 656 (3.4 per cent) had active lesions, 4.616 (24 per cent) showed the scars, and 13,917 were negative. The percentage of active lesions in the rural portion of the zone to be treated (Roseto, Morro d'Oro, Notaresco, Castellalto) was 3.3 per cent, in the rural test zone (Giulianova, Atri) 3.5 per cent.

It may be noted, from these results, that the incidence is apparently higher in the rural areas than in the towns or populated centers.

Combining the totals for the town and rural populations, out of 28,599 persons, 847 (2.9 per cent) had active lesions, and 5,943 (20.8 per cent) had scars. Thus a total of 6,790 persons, or 23.7 per cent of the entire population, were suffering from, or had had, cutaneous leishmaniasis.

EXPERIMENTAL PROPHYLAXIS

The first treatment with DDT was started in the summer 1948. The DDT spraying began on June 6th and was finished on August 18th, when the sandfly season was more than half over. Every house and stable within the territory of the four towns noted above was sprayed once during that summer with 2 grams of DDT per square meter, applied to walls and ceilings (an emulsion of the xylol-triton solution, containing 5 per cent DDT, was used).

During the spring (from March to May) of 1949 a second examination of the entire population was made in the treated as well as in the untreated districts. A second application of DDT was made during the summer (June 10th–July 31st) of 1949: it was not possible to do it earlier, although a pre-season application would theoretically have been desirable. A third examination of the entire population followed during the spring (from March to June) of 1950.

No definite catching stations for *Phlebotomus* were established. During the sandfly seasons the writer made almost continuous personal surveys of a great number of stables and houses all over both the treated and untreated districts.

After the first application of DDT an immediate and outstanding result was, with one observed exception, the complete disappearance of *Phlebotomus* inside houses and stables, while, on the contrary, they were found in great numbers inside the human and animal habitations in the untreated test zones. As described in a previous note (Corradetti 1949) on one occasion two and one-half months after the first application of DDT, it was possible to find *Phlebotomus* biting man outside as well as inside a certain house in the treated zone, but after the second treatment in 1949 no *Phlebotomus* was observed in the treated zone either indoors or outside. These results support the hypothesis advanced in the above noted paper that sandflies found in the treated zone in 1948 represented a newly emerged brood from eggs laid before the first spraying of DDT.

Special attention was paid to the transmission of oriental sore, as revealed by the second and third examinations of the population, following respectively the first and second treatment with DDT. (See graph.)

In the spring of 1949 there were examined 22,709 persons in the treated zone. Of these individuals, 161 (7 per thousand) were new cases of oriental sore, having been negative at the previous examination. In the untreated test zones the new cases were 88 out of 2,530 persons examined (34.7 per thousand). This examination showed that in the treated zone, new cases in the spring following the first treatment were proportionately only one fifth as numerous as in the untreated zones. Moreover, on account of the period of incubation, which may be very long, there is no evidence that the new cases in the treated area were contracted after the application of DDT.

In the spring of 1950, 25,220 persons were examined in the treated zone; there were 34 new cases of oriental sore (1.3 per thousand). In the untreated zones there were 101 new cases out of 2,449 persons examined (40.4 per thousand).

From the results of the third examination of the entire population, it can be concluded that the transmission of oriental sore for all practical purposes had been entirely interrupted in the treated zone. The very low proportion of 1.3 new

cases per thousand probably could not be eliminated unless the application of DDT were extended to the entire endemic region. It is obviously impossible to prevent the people living in the treated zone from contracting the infection when visiting the nearby untreated zones either to work or for other purposes during the season of transmission.

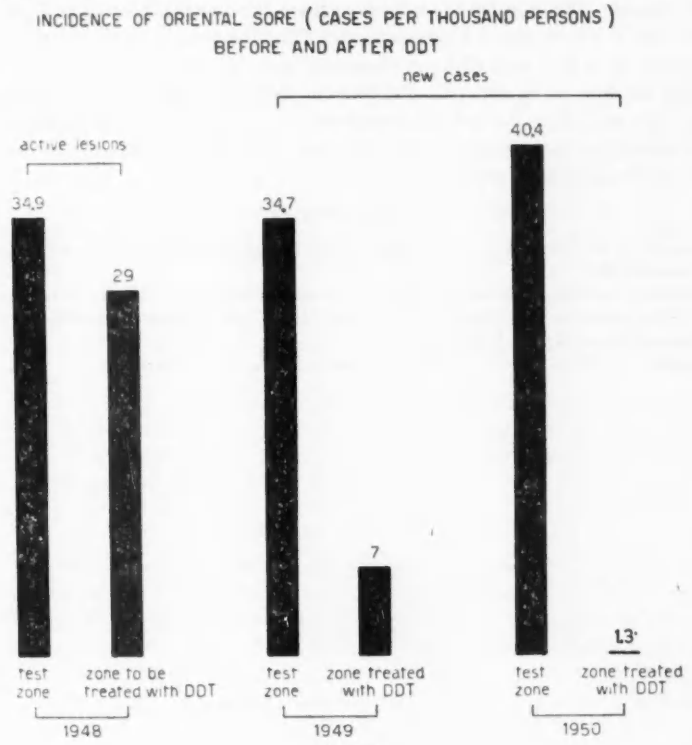

FIG. 2

The cost of this experimental prophylaxis was 160 Italian lire ($0.25) per person per year. This figure includes the cost of the DDT, the wages of workmen (3 spray crews of 7 men each) and transportation, but does not include the salaries of the directing staff.

The following additional notes may be useful to those wishing to undertake an extensive census of the sort described in this paper: 1) One doctor and two assistants constituted the census team. 2) The time required for each examination of the entire population was about two months. This length of time was necessary because of the hilly nature of the country, together with the fact that most of the rural houses are isolated, scattered all over the territory, and could be reached only by walking.

SUMMARY

An epidemiological investigation revealed that in a district of the Province of Teramo (Abruzzo, Italy), 23.7 per cent of the population showed either active lesions or the scars of oriental sore. Two subsequent annual applications of residual DDT inside houses and stables were sufficient to stop the transmission of the disease. The porportions of new cases in the treated zone were 7 per thousand after the first, and 1.3 per thousand after the second application of DDT, compared with 34.7 and 40.4 per thousand respectively in the untreated zones.

After the first DDT spraying *Phlebotomus* had disappeared, with an occasional exception, and after the second treatment no *Phlebotomus* at all was observed either indoors or outside in the treated zone, while these insects continued abundant in the untreated areas.

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IN VIVO SUSCEPTIBILITY OF *TRICHOMONAS VAGINALIS* TO ANTIBIOTIC THERAPY

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Recent published reports show *in vitro* susceptibility of *Trichomonas vaginalis* to the different antibiotics. When tested against the trichomonads in acidified serum, tyrothricin and tyrocidin have been effective in useful concentrations while gramicidin, streptomycin, hemopyocyanine and sodium penicillin were without effect (Trussell 1947). Greenblatt and West (1949, 1950) noted the superior trichomonicidal activity *in vitro* of aureomycin and tyrothricin to that of streptomycin, chloramphenicol or penicillin. McVay and his associates (1949, 1951) state that *in vitro* studies reveal that aureomycin is trichomonicidal and appears to be an effective therapeutic agent in *Trichomonas vaginalis* vaginitis. Greenblatt and Barfield (1951) find aureomycin, tyrothricin and terramycin to be the most effective agents *in vitro*, with the first proving most promising clinically.

The purpose of this communication is to present data on the effect of five antibiotics on *Trichomonas vaginalis in vivo*. These observations were made in an attempt to determine whether parenterally or orally administered antibiotics cleared the patients of their vaginal infestations.

MATERIAL AND METHODS

The study group was composed of female patients under antibiotic therapy in the outpatient and ward services of the Jefferson Medical College Hospital.

A wet-mount preparation of the vaginal discharge was examined before institution of the indicated therapy. All patients positive for *Trichomonas vaginalis* were then re-examined following completion of their treatment. With the exception of 14 pregnant patients attending the prenatal clinic, all were hospitalized.

RESULTS

Nine pregnant patients remained positive for *Trichomonas vaginalis* under luetic treatment with 600,000 units of penicillin daily for 10 days. Ten patients received dihydrostreptomycin, alone or with para-amino-salicylic acid (P.A.S.), with total dosages of the former ranging from 3 grams over a three-day period to as high as 40 grams over a forty-day interval, with no demonstrable diminution in numbers of vaginal trichomonads. The same result was obtained when terramycin, aureomycin or chloramphenicol were administered irrespective of total dosages employed.

Five normal, pregnant patients were given 250 mgm. or 500 mgm. of terramycin,* every six hours for seven to fourteen days; six patients received aureo-

* Supplied through the courtesy of W. Alan Wright, M.D., Chas. Pfizer & Co., Inc.

mycin, 250 mgm. or 500 mgm. every three hours or six hours for three to twenty-two days and eight patients were under chloromycetin therapy, 250 mgm. or 500 mgm. per sixth hour for five to twenty-one days.

In all of the cases treated with the five antibiotics, alone and in combination with penicillin, no effect could be observed on the trichomonads of the vagina. However, a favorable influence of the agents on the abnormal vaginal flora was apparent.

DISCUSSION

From time to time new forms of therapy are recommended to eliminate the presence of *Trichomonas vaginalis* in the vagina. Many agents and methods have been used but no one therapy is consistently successful. This failure to cure the patient is due to the high incidence of recurrence and reinfestation. Extravaginal foci of the parasite are responsible for the failure of treatment applied only to the vaginal mucosa. Involvement of the lower urinary tract (Stein 1933; Karnaky 1934; Pattyson 1937; Allen *et al.* 1946; Coutts 1951) and the glands of Bartholin and Skene (Shelanski *et al.* 1939) are sources of residual infestation for the vagina. The incidence of male infestation as a source of reinfestation of the female has been emphasized (Allen *et al.* 1935; Drummond 1936; Cornell *et al.* 1936; Karnaky 1938; Feo 1944; Freed 1948). Therefore, there will be no unanimity of therapeutic cures unless an agent is found capable of destroying the protozoon in all of its sites in the female and male. The choice of agent would be an orally or parenterally administered and absorbable drug.

The answer to this vexing problem appeared forthcoming with reports on the antitrichomonadal activity *in vitro* of the different antibiotics (8, 9, 10, 13, 14, 19). However, examination of these data on *in vitro* sensitivity determinations reveals that the criterion for trichomonicidal or trichomonistatic concentrations of the agents employed not infrequently is based on visible motility of the organism (Greenblatt *et al.* 1950). It is admittedly difficult to say whether a trichomonad is viable or not by noting its motility alone. Nevertheless, aureomycin has been reported to be effective clinically when applied locally as a powder (McVay *et al.* 1949, 1951; Greenblatt *et al.* 1951). Although McVay and his co-workers (1950) observed no destruction of *Trichomonas vaginalis* in women when oral aureomycin was administered in therapeutic amounts, they do report ten cases of male trichomoniasis successfully treated (1951).

A critical examination of the cases shows that in no instance were the trichomonads affected when the patient was under antibiotic therapy. Likewise, no effect could be demonstrated by the combined therapy. In contrast to this, a local and favorable effect on the abnormal vaginal flora was apparent by the orally or parenterally administered antibiotics.

SUMMARY

Five antibiotics, alone and in combination with penicillin, were under observation for *in vivo* effectiveness against *Trichomonas vaginalis*.

The five antibiotics were aureomycin, chloramphenicol, penicillin, dihydrostreptomycin and terramycin.

In this series of cases no effect on the vaginal trichomonads could be demonstrated by the antibiotics employed in therapeutic amounts.

ACKNOWLEDGMENTS

The author is indebted to Drs. Lewis C. Scheffey, Thaddeus L. Montgomery, Hobart A. Reimann, David M. Davis, Arno E. Town and Rudolph Jaeger for the facilities of their respective Services and wishes to express his grateful appreciation of the interest and advice of Dr. David H. Wenrich, University of Pennsylvania.

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THE EFFECT OF AUREOMYCIN ON BALANTIDIASIS[1]

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INTRODUCTION

During the past ten years various amebicidal and bacteriostatic compounds have been tried with some success in the treatment of balantidiasis. The compounds may be divided into four major groups: (a) arsenic compounds, such as Acetarsol (Liu, 1941; Diaz-Atiles, 1943) and carbarsone (Young and Burrows, 1943; Tsuchiya and Kenamore, 1945; Swartzwelder, 1950); (b) iodine compounds, as Diodoquin (Hummel, 1940; DeLanney and Beahm, 1943; Shookhoff, 1951), Enterovioform (Rens, 1948; Niño, 1948) and mercury biniodide (Shun-Shin, 1947); (c) sulfonamides, such as sulfathiazole (Hirvonen, 1947) and 2-phthalylsulfanilaminothiazol (Brummer, 1950); and (d) antibiotics, as bacitracin (Castellanos, del Prado, Garcia and Montero, 1950), aureomycin *in vitro* (Agosin, Christen and Rubio, 1950a) and *in vivo* (Neghme, Miranda, Agosin and Sanz, 1951) and terramycin *in vitro* (Agosin, Christen and Rubio, 1950b) and *in vivo* (Neghme *et al.*, 1951).

Prior to the appearance of the papers on the effect of aureomycin against *Balantidium coli* (Agosin, *et al.*, 1950a; Neghme, *et al.*, 1951), two cases of balantidiasis were encountered in the San Juan Area of Puerto Rico and treatment instituted. Four other cases were found later and this paper covers the treatment of the six cases with aureomycin. A seventh case was found prior to the initiation of the treatment program but was not used in this study.

PROCEDURE

Each patient had five to seventeen stool examinations made prior to treatment, nine to thirteen examinations during treatment and as many as possible during the post-treatment period. In that way an almost daily check was made up through the second to fourth post-treatment week.

In order to determine all species of parasites harbored, the following techniques were used on all specimens: saline smear, iodine smear, zinc sulfate flotation, 10% alcohol sedimentation and AMS III Technique. On representative specimens of each patient additional techniques were used, such as Quensel's stain, iron hematoxylin stain and culturing on Nelson's alcoholic extract medium, Cleveland's medium and Locke's egg medium.

Several types of media, either specific or modified, have been utilized with

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[4] Sincere appreciation is due Captain Howard E. Hall, MC, for administering the drug to the patients and to Mrs. Wilda B. Knight and SFC Rogelio Reyes for their technical assistance.

TABLE 1

Treatment of balantidiasis with aureomycin

PATIENT				PRE-TREATMENT PERIOD				TREATMENT PERIOD				POST-TREATMENT PERIOD			
					Stool examinations			TREATMENT		*B. coli*					
No.	Sex	Age	Wt.	Duration of diarrhea or dysentery	*B. coli*		Other parasites found	Total Gm.	Duration—Days	Day of last pos. spec. during treatment	Other parasites disappearing	Duration—Wks.	No. of examinations made	Other parasites not recurring	Remarks
					t	c									
1	M	22	145	Diar. 2 wks.	+	0	Eh, Ec, En, Cm, Gl, Na, Tt	28	14	3	Eh, Ec, En, Gl	2	10	Eh, Ec, Gl	Transferred from San Juan Area
2	F	11	?	Diar. ? 2–3 mos.	+	0	Al, Na, Ss, Tt	28	14	3	None	4	17	None	Discharged from Hospital
3	M	60	220	None	+	0	Na, Tt	17	11	3	Na	12	34	None	
4	M	25	155	Diar. 10 mos.	+	0	Eh, Ec, En, Gl, Na, Tt	22.5	14	3	Eh, Ec, En, Gl	11	31	Eh, Ec	
5	M	33	148	None	+	0	Ec, En, Gl, Na, Tt	20	13	4	Ec, En, Gl	5	23	Ec, En, Gl	Transferred from San Juan Area
6	M	20	120	Diar. 2 mos.	+	0	Eh, En, Gl, Na, Tt	21	13	3	Eh, En	10	30	Eh	

ABBREVIATIONS: Al—*Ascaris lumbricoides*, Cm—*Chilomastix mesnili*, Ec—*Endamoeba coli*, Eh—*E. histolytica*, En—*Endolimax nana*, Gl—*Giardia lamblia*, Na—*Necator americanus*, Ss—*Strongyloides stercoralis*, Tt—*Trichuris trichiura*, c—cyst, t—trophozoite.

varying degrees of success in the cultivation of *B. coli*. Nelson's alcoholic egg yolk extract medium proved to be an excellent medium for the isolation and maintenance of Puerto Rican strains of *Balantidium coli*, one strain having been maintained through 53 serial transfers. Egg and Cleveland's media proved less satisfactory than Nelson's in both isolation and maintenance. Further observations on the culture of this ciliate will be reported in a forthcoming paper.

RESULTS

Five of the six patients were prisoners at the Insular Penitentiary and the sixth was an eleven year old girl hospitalized at the Bayamon District Hospital. Three of the patients had *Endamoeba histolytica* in addition to *Balantidium coli*, so that the diarrhea could be attributed to a combination of the two. Two of the prisoners had no history of diarrhea and apparently were asymptomatic carriers. All patients harbored two to seven species of parasites in addition to *B. coli*.

Two patients received two grams of aureomycin daily for fourteen days or a total of 28 grams, with no complaints. The other four complained of various side effects of the drug, such as nausea and vomiting, and they received lesser amounts which varied from 17 grams during eleven days to 22.5 grams in a fourteen day period.

In all patients *B. coli* disappeared during the first four days of treatment and did not reappear (Table 1). Post-treatment examinations were continued for as long a period as possible and in no case was a relapse either of *B. coli* or *E. histolytica* seen.

Several other species of parasites disappeared during treatment of some patients but remained present in other patients. Those in whom they disappeared apparently had light infections, which showed up on few examinations. The only parasite, other than *B. coli* and *E. histolytica*, which failed to reappear in all patients originally harboring it was *E. coli*.

DISCUSSION

In examinations made of 544 Puerto Ricans, *Balantidium coli* was found in seven, or 1.3 per cent. This is double the incidence reported by Shookhoff (1951) in his examinations of approximately 3,000 Puerto Ricans living in New York City. However, six of the seven cases encountered in the San Juan Area were in prisoners at the Insular Penitentiary. Thus, in the group of 332 prisoners examined, 1.8 per cent harbored *B. coli*. This is in line with the greater prevalence of certain parasitic infections among institutional patients where sanitary conditions are low, as shown by Young and Ham (1941) who found seven *B. coli* infections in a group of 142 patients in a mental hospital.

Of the various drugs which have been used in recent years in the treatment of balantidiasis, several require further use before their effectiveness can be evaluated. Acetarsol has been reported used on four patients (Liu, 1941; Diaz-Atiles, 1943; DeLanney and Beahm, 1943), with one failure; Vioform on two patients (Rens, 1948; Niño, 1948) successfully; two sulfa drugs, each with suc-

cess on one patient (Hirvonen, 1947; Brummer, 1950), one of whom, however, required a second course of treatment; and bacitracin, which apparently was successful on two patients (Castellanos, *et al.* 1950), although the length of the follow-up period is unknown.

The three drugs which have been used on the largest numbers of patients are: (a) carbarsone on 13 patients (Young and Burrows, 1943; Tsuchiya and Kenamore, 1945; DeLanney and Beahm, 1943; Shookhoff, 1951), with nine being cured on the first course, two on the second course and two failures. Both failures (DeLanney, Shookhoff) might have been cured, had the dosages been doubled. (b) Diodoquin on nine patients (Hummel, 1940; DeLanney and Beahm, 1943; Shookhoff, 1951) with one failure, which was attributed to the fact that too small a dosage was administered (Shookhoff). (c) Mercury biniodide was administered to nine patients (Shun-Shin, 1947) and apparently was successful, although follow-up examinations were inadequate. One patient required two courses of treatment and later a mercury biniodide enema. Another was treated again about eight months later.

In the present study, which was begun prior to the appearance of the other papers (Agosin, *et al.*, 1950a; Neghme, *et al.*, 1951) on aureomycin, this drug was administered to six patients, three of whom had concurrent *E. histolytica* infections. The amount of drug varied from 17 grams during an eleven day period to 28 grams in two weeks. In all cases *B. coli* disappeared from the stool within four days and the symptoms were mitigated in the four showing pre-treatment diarrhea. Frequent post-treatment examinations for two to twelve weeks failed to show any recurrence of either *B. coli* or *E. histolytica*. This confirms the results obtained by Neghme and his co-workers, who found that the parasites disappeared during the second to fourth day of treatment. However, there was no lengthy post-treatment period of examinations given their patients. They used shorter and lighter courses of treatment than were used in this study and apparently were successful, whereas this investigation was conducted on a group of patients, half of whom also harbored *E. histolytica*, and a longer course was deemed advisable.

SUMMARY AND CONCLUSIONS

1. Six patients infected with *Balantidium coli*, three of whom also harbored *Endamoeba histolytica*, were treated with aureomycin and all remained negative for these infections in frequent post-treatment examinations of two to twelve weeks' duration.

2. In all cases *B. coli* disappeared within four days of initiation of treatment and it is probable that a shorter course of treatment might prove equally effective against this infection.

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ROCKY MOUNTAIN SPOTTED FEVER IN PANAMA

Report of Two Cases

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The purpose of the present paper is to report two more cases of Rocky Mountain spotted fever, which brings to four the total number of cases reported on the Isthmus of Panama. Both patients were treated with antibiotics and recovered. The diagnosis in both cases was confirmed by the complement fixation test.

REPORT OF THE CASES

Case No. 1—J. W., yellow race, female, 65 years old, was admitted to the Panama Hospital the 26th of January, 1951, because of a remittent fever of six days duration, with rhinitis, headache, nausea and vomiting.

The *physical examination* revealed a discrete maculo-papular rash on the lateral wall of the thorax, the abdomen, and the extremities. No petechial spots were observed. The tongue was coated and the breath was unpleasant. The liver was two fingerbreadths below the right costal margin upon inspiration and was not tender. Examination of the cardio-vascular system showed cardiac hypertrophy (the apex was in the sixth space, two centimeters to the left of the midclavicular line), due to hypertension (B. P. 210–110) and arterio-sclerosis. Its functional capacity was grade II. The rest of the physical examination was essentially negative.

Progress notes: On the third day of hospitalization (ninth day of illness) two or three petechial spots were noticed on the legs. The maculo-papular rash covered the entire body; only the face, the palms of the hands and soles of the feet were clear.

The temperature was of the remittent type; on the sixth day of the patient's hospitalization and thirteenth day of the disease, the patient had a normal temperature in the morning for the first time (Fig. 1). The physical examination at this time showed that the liver was still palpable, but not painful; the tongue was less coated and the breath was less offensive. The rash had persisted and it was observed that some spots, especially the ones on the lower extremities and some on the chest, did not disappear under pressure.

During the following seven days the patient had a temperature up to 99.6°F. in the evenings; in the mornings she was afebrile. Her general improvement was very slow in contrast with the febrile evolution.

Laboratory work: Thick smears for malaria taken at three different times during the first week of hospitalization did not show any parasites in the circulating blood. The red blood cell count was about four and a half million per cmm.; the white blood cell count was about ten thousand per cmm. with a

differential of 70% neutrophiles and 30% lymphocytes. Four agglutination tests, performed every other day with paratyphoid A & B, Typhoid O & H, *Proteus* OX19 and *Brucella abortus* antisera were all negative. An X-ray of the chest showed a moderate cardiac hypertrophy and dilatation of the left ventricle

CASE No. 1

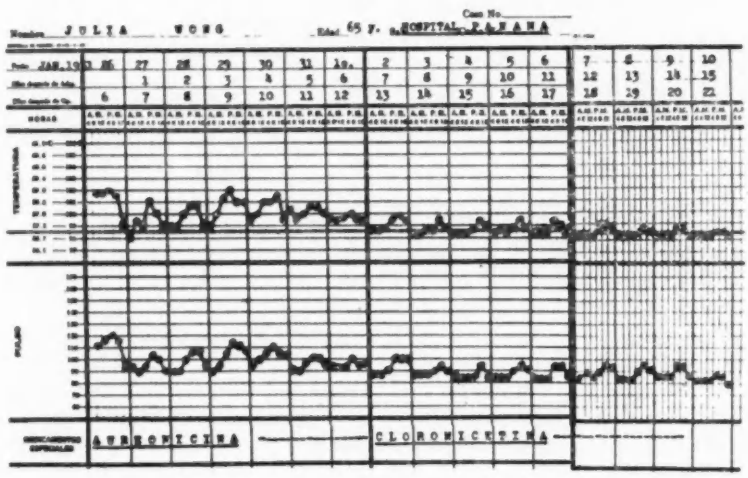

FIGURE 1

and a marked dilatation of the arch of the aorta. Several urine tests showed one to two plus albumin and a specific gravity between 1016 and 1024.

Agglutinations	*Jan. 30, 1951*	*Feb. 13, 1951*	*May 21, 1952*
Proteus OX19	0	1/40	0
Proteus OX-2	0	1/80	0
Proteus OX-K	0	0	0
Brucella	0	0	0
Tularemia	0	0	0
Rickettsia prowazekii	0	0	0
Rickettsia mooseri	0	0	0
Complement Fixation			
Dermacentroxenus rickettsii	1/16	1/64	1/16
Rickettsia conorii	0	1/8	0
Coxiella burnetii	0	0	0
Rickettsia akari	0	0	0

Treatment: From the 6th febrile day to the 9th day inclusive, the patient took 250 mg. of aureomycin every four hours. From the 10th febrile day to the 20th she was given 500 mg. of chloramphenicol every four hours. She received a total of 6 grams of aureomycin and 24 grams of chloramphenicol.

The *diagnostic* impression at the time of hospitalization was murine typhus,

but the serological test performed afterwards revealed that this was a case of Rocky Mountain spotted fever.

Case No. 2—E. M., mestizo, male, 28 years old, was hospitalized at the Santo Tomas Hospital on April 5th, 1951, with a remittent fever of four days duration, headache, generalized aching and a sensation of chills.

Physical examination was essentially negative with the exception of mild anemia and painless splenomegaly.

Progress notes: From that time and in a progressive form, a "typhus" appearance was evident, which, at the 9th febrile day was very marked. At this time he had, in addition, a cough and a maculo-hemorrhagic rash involving the

CASE No. 2

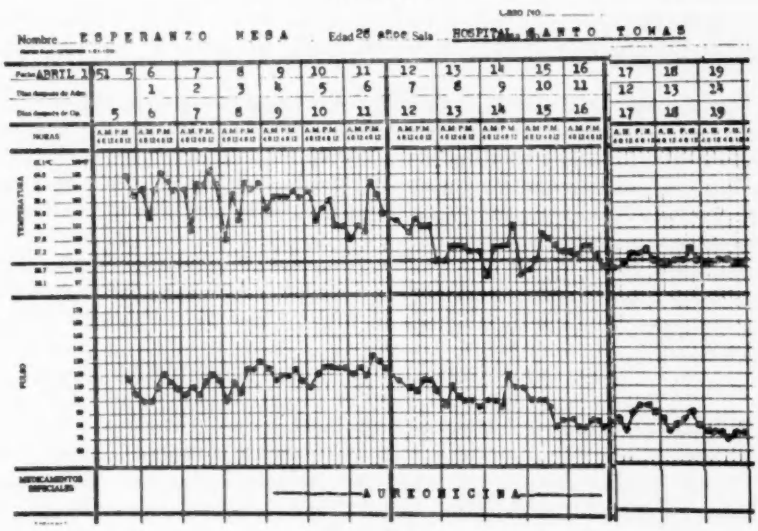

FIGURE 2

neck, chest, abdomen, and extremities, but sparing the face, the palms of the hands and the soles of the feet. Examination of the lungs revealed rales through out, but more marked at the bases; the heart examination revealed tachycardia, but no signs of cardiac failure. The rest of the examination was essentially negative.

On the 13th day of the illness the temperature returned to normal during the morning and rose to 99°F., in the evening. This was repeated the following six days. (Fig. 2)

Laboratory work: Five thick blood smears taken during the first days of hospitalization were negative for malarial parasites. A white blood count taken on the fifth febrile day showed 7,800 cell per cmm., with 58% neutrophiles, 2% basophiles, 35% lymphocytes and 5% monocytes. Two Kahn reactions, the

first taken on the fifth febrile day and the second on the 11th day of apyrexia, were negative. A blood culture on the fifth febrile day was reported sterile. The urine test showed 2 plus albumin during his febrile state and occasional red blood cells.

Four agglutination tests with Paratyphoid A & B, Typhoid O & H, *Proteus* OX19 and *Brucella abortus* antisera (taken on the 9th and 13th febrile days and on the first and 9th day of postapyrexia) were all negative.

Agglutination	*April 14, 1951*	*May 4, 1951*	*March 3, 1952*
Rickettsia prowazekii	0	0	0
Rickettsia mooseri	0	0	0
Complement Fixation			
Dermacentroxenus rickettsii	1/64	1/256	1/512
Rickettsia conorii	0	1/8	0
Coxiella burnetii	0	0	0
Rickettsia akari	0	0	0

The *diagnostic* impression at the time of hospitalization was malaria. When the typhus state and the rash were evident, the diagnosis was thought to be murine typhus. In this case, however, the clinical diagnosis of *Rocky Mountain spotted fever* could be made because of the localization of the exanthema, which was most marked about the wrists and ankles. The complement fixation test confirmed the clinical diagnosis.

Treatment: From the 9th day of illness, as soon as the diagnosis of rickettsiosis was suspected, the patient took 500 mg. of aureomycin every four hours, until the temperature returned to normal in the evenings. He received 25 grm. of aureomycin in all.

COMMENT

History: The medical bibliography at hand shows that on the American Continent Rocky Mountain spotted fever was first diagnosed in the United States of America (Maxey 1899) and that later on it was also recognized in Mexico (Bustamente *et al.* 1943), Brazil (Piza *et al.* 1932), Colombia (Patiño *et al.* 1937) and Canada (Gibbons 1942). It seemed probable that the disease might exist in other American countries where the vector or vectors were present; nevertheless, due to the lack of laboratory facilities, the existence of Rocky Mountain spotted fever in Panama was not established until recently, when Rodaniche and Rodaniche (1950) reported the first two proven cases. In the first case, the diagnosis was based on the isolation of a rickettsial strain from the blood obtained before the patient's death and by characteristic serological reaction; and in the second case, it was based on the complement fixation test. With the two new cases reported in this communication, the cases diagnosed and confirmed by laboratory procedures total four.

Prognosis: Even though the number of cases seen is very small, it is gratifying to note that a cure resulted in the three cases in which the diagnosis of rickettsiosis was suspected and specific treatment was instituted. Only the first of the 4 cases diagnosed died; the diagnosis was made post-mortem, and no antibiotic

treatment was prescribed. Nevertheless, we believe that the prognosis of the disease here is the same as in other countries. In the case in which no antibiotics were used, death ensued while in the other three cases, due to the specific antirickettsial action of the antibiotics, the patients recovered.

Source of infection: In the first case, the epidemiologic survey showed that the patient was of advanced age, and that she spent her life in her home located in the city, opposite the Government Palace. We found a dog living in the same house covered with ticks collected on its periodic excursions. We followed this lead hoping to establish the source of infection. Thirty larvae, nymphs, and adult ticks, of the species *Rhipicephalus sanguineus* were collected and injected into several guinea pigs. The results were negative. It should be noted, however, that we collected the ticks three months after the patient was in the hospital and that ten weeks earlier the dog had been taken to a veterinary hospital for a germicidal bath. It is possible that this bath killed any infected ticks that may have been present.

In the second case the cycle of infection could be explained by the fact that the patient was a farmer and that he had to spend many hours of the day in contact with ticks, which are possible carriers of the rickettsia. The patient gave his address as the Trans-Isthmian Highway, near the Cement Factory. We have taken larvae, nymphs, and adult ticks of dogs, horses, cattle and from the grass of the yard of this second patient to inoculate guinea pigs and will report our findings at a later date.

Diagnosis: From the *clinical point of view*, the diagnosis in the second case could be made after the 10th febrile day with the localization of the rash, which was most marked on the wrists and ankles; by the petechial character of the same; by the typhus like picture frankly manifested, and by the agglutination tests which were negative with *Proteus* OX19 antigen. In the first case, on the contrary, the rash was relatively moderate with only a few petechial spots which were without characteristic localization, in spite of the fact that the typhus-like state was frankly manifested, although not as clearly as in the second of the reported cases. The diagnosis of *rickettsiosis* was made in this first case, but we did not have in mind the clinical entity which the laboratory reported. The clinical diagnosis in each of the cases was confirmed by the complement fixation test performed with the technique used by the Virus and Rickettsia Laboratories of Mexico and the Rocky Mountain Laboratory in the United States of America, both of which employ antigen obtained from egg embryos infected with strains of *Rocky Mountain spotted fever*. In both cases at least two specimens of serum were taken at different times in the evolution of the illness. An increase in the titer of the antibodies was observed which corroborated the diagnosis of spotted fever in both patients.

Geographical distribution of spotted fever on the Isthmus of Panama:

Due to the fact that the first two cases with spotted fever came from the vicinity of Chorrera, the third from the Trans-Isthmian Highway, and the fourth and last case from the City of Panama, we believe that the disease is spread

over the Isthmus. With this in mind we have started a study of the epidemiology of the disease in this country. We have taken ticks from different places on the Isthmus for inoculation into guinea pigs, attempting to produce the infection in animals and isolate the *rickettsia*.

At the same time we have taken blood from people from different parts of the country representing most of the communities of the Isthmus. Although we have not finished this work, the preliminary findings are interesting, and this will be the subject of another report.

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PRELIMINARY OBSERVATIONS ON THE PREVALENCE, CLINICAL MANIFESTATIONS AND CONTROL OF FILARIASIS IN THE SOCIETY ISLANDS

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INTRODUCTION

Although Stoll (1947) estimated that 189 million people throughout the world are infected with filariasis, little effort was directed toward its control prior to World War II when an increased interest was developed in the disease as a result of the great number of the armed forces who acquired filariasis in the South Pacific (King, 1944).

Early investigations by Bahr (1912), O'Connor (1923), and Buxton (1927), had established its occurrence in the Pacific Islands but probably the first extensive attempt to organize a control program against filariasis in the South Pacific was by Amos (1947) in Fiji who emphasized sanitation only. Later, control measures were instituted in Rarotonga by Davis (1949). Byrd *et al.* (1945) made a preliminary study in American Samoa and later Wharton and Jachowski (1950) began a research and control program in which they studied the effects of sanitation procedures, DDT and drug therapy either separately or in combination.

The first survey was begun by Mr. Robinson in 1947 and the results of this and additional studies in the district of Paea were first reported by Galliard, Mille and Robinson (1949) in which 30 per cent of the inhabitants were found to show microfilariae in the peripheral blood. Nineteen per cent of the series gave a history of lymphangitis and 9 per cent of elephantiasis. The first control meas

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[5] On August 28, 1947, after two years of preliminary investigation, Mr. Wm. A. Robinson of Tahiti proposed to the Governor of French Oceania through Dr. M. Bonnaud, then Chief Medical Officer, that he contribute funds to help found a medical research institute in Tahiti which would provide facilities for international collaboration in the study and prevention of tropic diseases in the Pacific and of filariasis in particular. The Institut de Recherches Médicales de l'Oceanie Francaise was subsequently created by the French Government and a cooperative scientific program designated as the Filariasis Research Project was developed at the University of Southern California, with Mr. Robinson and Mr. Cornelius Crane as founding members. In 1951 the cooperative program was expanded and transferred as the Pacific Tropic Diseases Project, to the Department of Infectious Diseases, School of Medicine, University of California, Los Angeles.

ures were begun in 1948 in Paea, when the combined group of workers from the Institut de Recherches Médicales de l'Oceanie Francaise and from the University of Southern California began their cooperative studies.

It is not known when filariasis was introduced into the Society Islands but Cook in 1774 (Lloyd, 1949) mentioned a case among the aborigines. Ellis (1833) in speaking of the Polynesians states that "elephantiasis appears to have prevailed from their earliest antiquity". Thus it seems highly probable that this disease was introduced by the Polynesians when they settled the islands long before the Europeans first visited the area.

Many compounds have been tested against filariasis to determine their therapeutic value. These have included suramin (Bayer 205) by Van Hoof *et al.* (1947), Burch (1949); ethylstilbamine (Neostibosan) by Culbertson *et al.* (1946, 1947), Culbertson (1948); lithium antimony thiomalate (Anthiomaline) by Brown (1944), Brown *et al.* (1946), Culbertson *et al.* (1946) and others, and all have shown little promise of being suitable for mass treatment of filariasis. More recently, Otto and Maren (1948) and Thetford *et al.* (1948) have reported considerable success with arsenamide but this drug has not been used on a large series of patients. Each drug thus far has exhibited the disadvantages of being either too toxic, too difficult to administer or of no value in reducing the clinical manifestations of the disease. However, Hewitt *et al.* (1947, 1949, 1950), Santiago-Stevenson *et al.* (1947), Galliard and Mille (1949), Hawking and Laurie (1949) Kenney and Hewitt (1949) and Oliver-González *et al.* (1949) have shown that 1-diethylcarbamyl-4 methylpiperazine (Hetrazan, Notezine), when given orally reduces the concentration of microfilariae in capillary blood. This drug is of low toxicity in laboratory animals (Harned *et al.* 1948), and is well tolerated by man, although a few minor complications have been observed (Galliard and Mille 1949; Kenney and Hewitt 1949; Hewitt *et al.* 1950, 1950a).

The purpose of the study presented here was twofold. The first objective was to learn more concerning the epidemiology and extent of the disease in the Society Islands, particularly on Tahiti and on Maiao. This included the prevalence of persons with microfilariae, filarial lymphangitis, elephantiasis or other complications which might identify the disease. Data pertaining to the species of mosquitoes, their prevalence, breeding habits and filarial infection rates also were collected. The second major objective was to compare the relative efficiency of several proposed methods for controlling the transmission of the disease. With the advent of promising microfilaricidal drugs and advancement in insecticide control, it was desired to learn whether any one of the drugs or mosquito control measures would be practical in reducing transmission, with ultimate eradication of the disease. The methods of control chosen for testing included combinations of diethylcarbamazine medication, mosquito sanitation and use of DDT. Although studies of certain phases of the project are incomplete, it is thought that results of observations gathered over periods from 12 to 24 months between November 1948 and September 1950 should be reported at this time.

MATERIALS AND METHODS

Data concerning the incidence and clinical manifestations of filariasis presented in this report were collected from ten experimental areas where several combinations of methods for the control of filariasis were to be tested. Seven of the areas were on the side of Tahiti with the greater rainfall; two areas were in Paea, having lesser rainfall, where Galliard *et al.* (1949) had conducted preliminary tests; and one area was on the island of Maiao.

DESCRIPTION OF TAHITI AND MAIAO

Tahiti is the largest and most important of the Society Islands, which are located in the South Pacific between 149° and 154° longitude and between 16° and 18° south latitude. It is in the shape of a figure 8 and is of volcanic origin with its highest peaks rising to more than 7,000 feet. The island is surrounded, except on the north and east, by a coral reef and thus lies in a lagoon. It is for the most part mountainous with narrow coastal plains, particularly along the west side. The climate of Papeete, the capital, is mild with a mean annual temperature of 80°F., an average humidity of 76% and an annual average of 78 inches of rainfall (Japy 1948). The remainder of the island varies considerably in rainfall and wind direction. The coastal portion of the island is roughly divided into dry and wet sides with the average annual rainfalls ranging from 70 to 150 inches respectively. Annual rainfall in the mountains, where there is precipitation almost daily, is not known but probably would exceed 400 inches in some areas. The island is roughly 105 miles around and covers about 600 square miles.

In 1949 there were approximately 35,000 inhabitants with some 15,000 living in the capital of Papeete. Tahitians are for the most part a mixture of Polynesian with either European or Chinese blood. Some 5,000 Chinese and approximately 2,000 Europeans also inhabit Tahiti.

The rural portion of the island is divided into 19 districts and the people live on both sides of a road which extends most of the way around the island. Homes as a rule are surrounded by neat yards, which are shaded by varieties of trees and shrubs. These include coconut, banana, mango, mape, breadfruit, false coffee, hibiscus and many other tropical plants. Numerous rivers and streams carry the vast rainfall through lush valleys to the ocean. The principal industries are the production of copra and fishing.

Maiao is a much smaller island, also of volcanic origin, and its one small mountain rises to a height of approximately 900 feet. It is surrounded by a reef which is impassible to all vessels except small craft such as canoes and life boats. Most of the island is but a few feet above sea level with several land-locked salt water lakes and marshes, and the terrain is covered with coconut plantations. It is inhabited entirely by Polynesians and there are no business houses. The chief industry is copra production. Accurate weather reports are not available.

EXPERIMENTAL AREAS

Part of the people selected for study lived in small communities, designated as areas I to IX, Figure 1. In general, the inhabitants were a cross section of the rural population, predominantly Tahitian, being a mixture of Polynesian and European blood except for two Chinese families in area VII. At the beginning of this study in areas I to VII, there were respectively 11, 14, 7, 13, 11, 9 and 8 inhabited houses. The number of persons living in each house ranged from 1 to 22 with a total of 401 inhabitants, or 5.5 persons per house. The European, American and Chinese population in Paea, i.e., areas VIII and IX, was slightly greater than the foreign population in the other areas. There were 96 houses in Upper Paea inhabited by 422 persons, or an average of 4.4 persons per house. The average number of persons per house in Lower Paea was similar

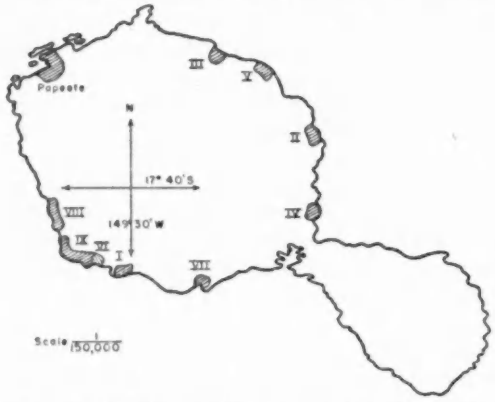


FIG. 1. Diagram of Tahiti showing the location of experimental areas I to IX.

to that in Upper Paea. There were 23 houses on Maiao inhabited by a total of 184 persons, or an average of 8 persons per house.

All localities had essentially the same physical properties, the land being flat or sloping gently toward the ocean. The mountains, however, were closer to areas III, IV, V and VI and at certain points bordered the lagoon. Areas II, VIII and IX also were close to the lagoon but the mountains for the most part were two hundred or more yards inland. Areas I and VII were in flat country approximately one-half mile from the mountains and several hundred yards from the lagoon. Houses ranged in size and structure from the simplest single-room native bamboo or palm frond hut, to more elaborate three or four-room native huts or open European style wooden frame homes. The yards were shaded with varieties of plants previously named. With the exception of Maiao, the animal populations in each of the areas were about the same. They consisted chiefly of dogs, cats, cattle, pigs, horses, a few goats, numerous chickens, a few ducks, many mynah birds, wild rats and lizards. There were no cattle or horses on Maiao. With the exception of the two areas in Paea and the village on Maiao,

the experimental areas ranged in size from about 300 to 500 yards long by 100 to 200 yards wide. The Paea areas were two and three miles long, and on Maiao the area extended for more than half a mile. Each area was mapped to show the location of houses and essential physical features. A census was made of all persons, by houses. The data included the name, sex, age and history of lymphangitis, acute filarial fever, varicocele, hydrocele, enlarged epitrochlear glands and elephantiasis. Also, the blood of each person over one year of age was examined for microfilariae in areas I to VII, of all but six persons over 3 years old on Maiao and of all persons 5 years or older in Paea. Additional information collected included the number of years a subject had lived in his district, household contact with elephantiasis cases and how long each subject had slept under a mosquito net. Representative numbers of animals were examined for microfilariae. Precontrol data for areas I to VII were gathered during July and August 1949, and control measures were begun in September of that year.

In addition to the seven areas on the wet side of the island, three other areas were studied. Two of these were in Paea and are designated as Lower and Upper Paea, i.e., areas VIII and IX respectively. These areas were much larger and included many more inhabitants than areas I to VII. The terrain, vegetation, etc. were essentially the same but there was less rainfall, particularly in Lower Paea, than in other areas. The district of Paea had been surveyed by Galliard *et al.* (1949) in the summer of 1948, and 51 of the inhabitants in Upper Paea had been given diethylcarbamazine in doses approximating 25 mgm. three times a day for 7 days. Preliminary mosquito control measures also had been instigated. Extensive control measures in Upper Paea were not begun until November 1948 and in Lower Paea not until January, 1949. The last area, designated as number X, was on the island of Maiao, which is located approximately 60 miles west of Tahiti. Diethylcarbamazine was administered in June, 1949. No mosquito control measures were undertaken.

CLINICAL EXAMINATION AND LABORATORY PROCEDURES

Examination of the blood for microfilariae was made by drawing a carefully measured 20 cmm. sample of capillary blood in the afternoon and placing it as a thick film on a clean slide. Each smear was allowed to dry, was then dehemoglobinized, fixed in 100 per cent methanol for 5 minutes and stained with a 1:20 dilution of Giemsa for 10 to 20 minutes. The numbers of microfilariae were then counted by two different persons. To aid in making accurate counts a Whipple disc, or homemade disc with two parallel lines about 7 mm. apart, was placed in the ocular and served as a guide while the operator searched systematically back and forth across the film for embryos. The blood of the persons in areas I to VII was examined before the experiment was begun and again at 3 months, 6 months and at 12 months thereafter. The post-treatment blood examinations on Maiao were made at 2, 10 and 14 months respectively. The intervals of the blood examinations in Paea are indicated later.

The subjects were given a brief physical examination by the senior author before administration of diethylcarbamazine and again at 6 and 12 months

thereafter except in area VIII. Special laboratory analyses were made on some of the subjects in Upper Paea, Lower Paea and in Maiao before and at periodic intervals after treatment. In Upper Paea, these included a urinalysis consisting of determination of specific gravity, pH, presence or absence of albumin and sugar and a microscopic examination. Blood differentials, hemoglobin and total red and white blood counts were also made. Stools of some of the subjects in Maiao and Lower Paea were examined for helminth ova to determine the anthelmintic activity of diethylcarbamazine.

During the period of the experiment, the number of attacks of lymphangitis that each subject suffered during each month was recorded and any new cases of elephantiasis were noted. As often as possible patients were visited during attacks.

Because of the migratory habits of the island peoples and the effect that such migrations might have on the transmission rate in a given area, a careful record was kept of those who visited or left each area for three days or more. Also any large gatherings of people in the areas were noted.

OBSERVATIONS ON MOSQUITOES

Except in Upper Paea, each area was carefully surveyed for all mosquito breeding places prior to instituting the experiment. Thereafter, with the exception of the island of Maiao, which was too distant for regular collections, each area was resurveyed at monthly intervals. Maiao was surveyed for mosquito breeding at 2 and 10 months post-treatment. Breeding places were plotted on maps of each area and designated by symbol as to type of breeding, location and the genus of mosquito found.

Adult mosquito populations were determined by making observations at three resting stations and three biting stations in each of the areas I to VII. Stations were selected as uniformly alike in all areas as possible. Three recordings were made during a period of 2 to 4 weeks prior to the control measures and at semimonthly intervals thereafter. All mosquitoes caught were counted, classified as to species and recorded. Collections at biting stations were made between 1 and 2 P.M. and all mosquitoes attacking the human bait during the first 10 minutes were caught and counted. Collections at resting stations were made between 8 and 9 A.M. No stations were established on Maiao and the 16 stations in Upper and Lower Paea were not established until November and December of 1949.

Lots of mosquitoes were collected, dissected and examined for filarial stages prior to control measures, excepting in Upper Paea. Similar lots of mosquitoes were again collected and dissected at three-month intervals thereafter in all areas with the exception of Maiao, where mosquitoes were captured for dissection before treatment and at 2, 10 and 14 months post-treatment. Adults were captured either while biting or while resting inside houses or within 10 meters of houses. Mosquitoes were collected in test tubes, brought to the laboratory and usually dissected the same day. When it was necessary to keep them for a longer period, the cotton plugs were moistened and the tubes were placed

in the refrigerator, which usually kept specimens alive and in excellent condition for dissecting. Each mosquito was dissected by separating the head, thorax and abdomen. Each portion was placed in a separate drop of saline on a slide, carefully teased apart and then examined for all stages of larvae. The number of advanced stages, and sometimes earlier stages, was counted and the presence of all stages was recorded.

OTHER OBSERVATIONS

Before and during the early weeks of the study, representative mature animals in each of the areas were examined for microfilariae to determine whether such animals might serve as sources for filarial infection in mosquitoes. Animals examined included dogs, cats, pigs, horses, cows, goats, chickens, ducks, rats, mynah birds and lizards. Samples were not measured but usually consisted of more than 20 cmm. of blood. Smears were handled and studied in the same manner as for human blood except that the exact numbers of microfilariae were not counted.

Weather stations were maintained under the supervision of the government meteorological service in or near each of the areas, except Maiao. Daily rainfall, mean temperature, temperature range, wind velocity and direction, and sunshine were recorded. In addition, weather records for the past two or three years were made available.

The indices used to determine the effect of the several control measures on the people and on area transmission were as follows:

1. Percentage of persons positive for microfilariae before and after treatment.
2. The average number of microfilariae per 20 cmm. of blood per person.
3. The frequency distribution of microfilarial counts.
4. The number of attacks of lymphangitis per month per person.
5. Larval mosquito density.
6. Adult mosquito density.
7. The filarial rate in mosquitoes.

In areas in which only DDT or sanitation was employed, it is obvious that no conclusions can be drawn within a relatively short period of time concerning the influence of these measures on the control of the disease. However, a comparison of mosquito populations and the average number of infective larvae in mosquitoes before and during the course of the experiment should provide information concerning differences in the transmission rate.

Control measures applied to each of the areas are shown in Table 1. Diethylcarbamazine was given to subjects at the rate of 2 mgm. per kilogram of body weight three times a day for seven days. It was administered by individual dosage under supervision of the Field Director and Field Campaign Director and patients were seen daily by one of the staff during and for two weeks following the course of administration. They were then checked at monthly intervals for attacks of lymphangitis and were given a physical examination at six months and at one year. Sanitation consisted of cleaning up or destroying coconuts, tin

cans and other mosquito breeding containers or places for a radius of 50 to 75 yards around each of the homes in an area. This also included some drainage, emptying or closing of tree holes and cutting of tall weeds and unwanted grass. Five per cent DDT emulsion in water was applied to the interior of the homes and sheds once every three months. The exterior surface of buildings and all shrubs within 20 to 30 yards of the houses were sprayed with one per cent DDT. Interior spraying was done with cylinder hand sprayers and exterior surfaces were sprayed from a jeep equipped with a power-take-off type sprayer.

TABLE 1

Number and percentage of persons positive for microfilariae in experimental areas before the inauguration of control measures. Tahiti and Maiao, 1948-50

AREA NUMBER	CONTROL MEASURES USED	NO. EXAMINED	PERSONS POSITIVE FOR M/F BEFORE CONTROL MEAS.	
			Number	Percentage
I	None	70	27	38.6
II	Treatment* of microfilaria positives	56	17	30.4
III	Treatment of everyone	37	17	45.9
IV	Sanitation only	51	15	29.4
V	Treatment of positives & sanitation	66	22	33.3
VI	DDT only	36	21	58.3
VII	Treatment of positives & DDT	60	19	31.7
Total for areas I to VII		376	138	36.7
VIII	Sanitation & DDT	302	107	35.4
IX	Treatment of positives & sanitation & DDT	422	119	28.2
X	Treatment of positives and of an equal number of negatives	165	43	26.1
Grand total		1265	407	32.17

* Diethylcarbamazine (Hetrazan).

RESULTS AND DISCUSSION

Prevalence of clinical filariasis

To obtain data concerning the prevalence of filariasis, physical examinations were made and histories taken on 369 subjects in areas I to VII and on 81 persons, or 40 per cent of the subjects on Maiao, area X. In addition to the demonstration of microfilariae, a history of lymphangitis associated with at least one of the following criteria was considered diagnostic of clinical filariasis:

a. Elephantiasis.

b. Palpable epitrochlear nodes in persons over ten years of age.

c. Varicocele, hydrocele or greatly enlarged femoral nodes.

One hundred eighty-four or 41 per cent of these Tahitians showed clinical signs of filariasis while an additional 67 or 15 per cent were asymptomatic carriers. The distribution of clinical signs varied with age and sex, the most interesting

TABLE 2

Number of persons examined and the number of persons positive for microfilariae, by age groups, experimental areas I to VII. Tahiti, 1949

AGE IN YEARS	NUMBER EXAMINED	NUMBER POSITIVE FOR M/F	PER CENT POSITIVE FOR M/F	AVERAGE NO. M/F PER POSITIVE
1–4	42	2	4.8	91.5*
5–9	55	13	23.2	77.9
10–19	78	26	32.1	91.8
20–29	78	35	43.9	113.7
30–39	58	22	37.9	103.7
40–49	36	19	52.8	268.1
50–Over	29	21	72.4	137.0
Total	376	138	36.7	127.7

* This figure is high for the average number of m/f for this age group as shown by subsequent examination of 1 to 4 year old positives in the other areas.

TABLE 3

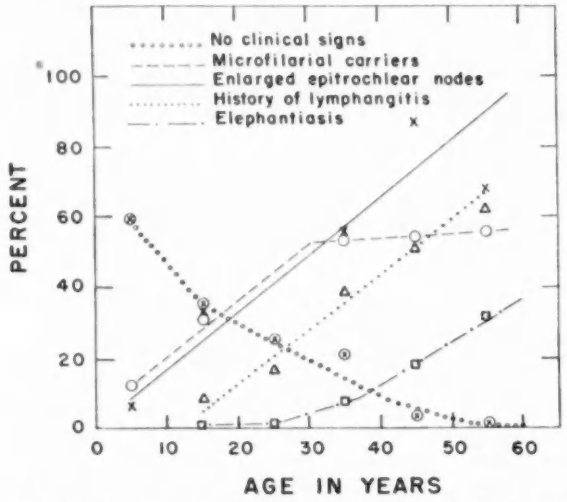
Prevalence of Bancroftian filariasis in male Tahitians. Number and age specific rates for clinical signs. Experimental areas I to VII. Tahiti

FACTORS	AGE GROUP											
	0–9		10–19		20–29		30–39		40–49		50 plus	
Number persons in each age group	47		37		43		32		26		18	
	No.	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
Enlarged epitrochlear nodes	3	6.5	12	32.4	16	37.2	18	56.1	22	84.5	12	67.0
Enlarged femoral nodes	4	8.5	13	35.1	17	39.6	14	44.0	17	65.5	9	50.0
Enlarged inguinal nodes	13	27.6	13	35.1	13	30.1	12	37.6	13	50.0	8	44.3
Hydrocele	0	0.0	0	0.0	4	9.3	5	15.6	7	27.0	4	22.2
Other signs (excluding elephantiasis)	1	2.1	3	8.2	3	6.9	6	18.7	7	27.0	3	16.6
No signs	28	59.5	13	35.1	11	25.6	7	21.9	1	3.8	0	0.0
History of lymphangitis	0	0.0	3	8.2	7	16.3	12	37.6	13	50.0	11	61.2
Census and blood survey (Data from 15 districts) Number of persons in each age group	751		870		549		519		357		344	
	No.	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
Positive for microfilariae	86	11.4	274	31.4	251	45.7	281	54.1	197	55.0	196	57.0
With elephantiasis	0	0.0	3	0.3	10	1.8	43	8.3	70	19.6	110	32.0

facts being: that no male was free of signs after the age of 50; that the percentage of palpable epitrochlear nodes was 41 per cent for males and 20 per cent for females, a difference that is not explained by age distribution; and that no single sign serves as an index of prevalence. The age specific rates are higher in the

older age groups for each sex. An example of the distribution of these signs for the male population is presented in Table 3 and Graph 1.

A group of 254 Tahitians who did not receive diethylcarbamazine were followed at monthly intervals for a year to obtain data on the prevalence and frequency of attacks of lymphangitis in a Tahitian population normal in respect to age, sex, and geographical distribution. Of these 254 Tahitians, 37 gave a history of previous attacks of lymphangitis, and of these 37, 19 experienced attacks during the year of observation while 18 did not. In addition, 4 persons experienced attacks for the first time during the year. The 23 Tahitians having lymphangitis had a total of 80 attacks. In terms of the total population, the



GRAPH #1 Age Specific Rates Among Tahitian Males for (1) Microfilaria Carriers*, (2) Enlarged Epitrochlear Nodes**, (3) History of Lymphangitis**, (4) Elephantiasis*, and (5) Without Signs of Filariasis**; Tahiti, 1949-1950.

* Data from basic prevalence survey in 15 of 19 Districts.

** Data from a sample of 203 Tahitian males in Experimental Areas I to VII.

attack rate was 0.34 attacks per person per year while in terms of those having attacks, the average number of attacks was 3.5.

Prior to the institution of control measures in experimental areas I to X, blood surveys revealed a high percentage of persons positive for microfilariae. Results are shown in Table 1. The figures for areas I to VII are based on an examination of most of the population one year of age and older, and show that 138 or 36.7 per cent, of 376 persons in areas I to VII were positive for microfilariae. Of persons five years of age and older in Lower and Upper Paea, 35.4 and 28.2 per cent respectively were positive. Of persons three years of age and older on Maiao, 26.1 per cent were positive. The total percentage of positives for these areas ranged from 26.1 to 58.3. Of those 5 years of age and older in all areas, an average of 40.7 per cent were positive in areas I to VII as compared

to 30 per cent as previously reported for Paea (Galliard *et al.* 1949), or the 31.8 per cent reported for Paea in this study, and 26.5 per cent on Maiao. As shown in Table 2, the specific rates of persons positive for microfilariae increased with age. Twenty-one, or 72.4 per cent, of 29 persons 50 years of age and older were positive for microfilariae. Positive adults averaged more microfilariae per individual than children, with the highest number of microfilariae per person being in the 40 to 49 year age group. The youngest positive was a 14 month old baby. The greatest concentration of microfilariae seen in a patient was in a 42 year old male who had 1094 microfilariae in 20 cmm. of blood. The data for areas I to VII, with minor exceptions, are in close agreement with the findings in Paea and on Maiao and for all the district populations in Tahiti. Of 364 positive persons in areas I to X whose microfilariae were counted, there was a gradual average increase in the number of microfilariae with advancing age.

In areas I to VII there were 211 males and 165 females, or 56.1 per cent and 43.9 per cent, respectively. Of the males, 39.81 per cent were positive as compared to 32.73 per cent of the females. These data are in fairly close agreement with those for Paea (Galliard *et al.* 1949). However, on Maiao where there were 107 males as compared with 77 females, a nearly equal percentage of each sex examined was positive, 26.6 and 25.4, respectively. There was a significant difference in the microfilarial concentration between sexes. One hundred seventy-one males had an average count of 130.4 microfilariae per 20 cmm. of blood as compared with an average of 66.7 for 115 positive females. It is of interest that approximately 50 per cent of the population was under 20 years of age.

The age relationship for certain of the clinical signs among male Tahitians is illustrated in Table 3 and in Graph 1. Data in this table and graph on age, specific microfilarial rates and elephantiasis were obtained from the island-wide census and survey made in 1949, and utilized in this instance because of the advantage of employing larger numbers.

Mosquito observations: Larval surveys and adult biting and resting collections made in areas I to IX during the experiment showed nine species of mosquito to be present in greater or lesser numbers in most areas. The species encountered included *Aedes pseudoscutellaris*, *A. tongae*, *A. aegypti*, *Aedes edgari* (Stone and Rosen), *Culex quinquefasciatus*, *C. atriceps*, *C. annulirostris*, *C. litoralis* (probably) and *C. sitiens*. Only three species, *A. pseudoscutellaris*, *C. quinquefasciatus* and *C. annulirostris*, were encountered on Maiao. Only *A. pseudoscutellaris* and *C. quinquefasciatus* were found consistently in large numbers as adults in all of the areas, the former being more numerous than the latter. *C. atriceps* was encountered in a high percentage of larval collections, but adults were not numerous. The next two most common species were *C. annulirostris* and *A. aegypti*. Distribution of the latter was particularly spotty. The remaining species were not commonly collected in or near homes.

Larval surveys revealed that the principal breeding sites of mosquitoes in Tahiti listed in their probable order of importance prior to instituting control measures were as follows:

1. Coconuts, rat-eaten.

2. Ground pools, ponds, swamps and ditches.
3. Pirogues (outrigger canoes).
4. Tree holes.
5. Tin cans, bottles, etc.
6. Coconuts, man-opened.
7. Fallen coconut leaf and flower axils, fallen banana and breadfruit leaves.
8. Drums, gasoline.
9. Tires.
10. Rock holes.
11. Kitchen drains.
12. Bamboo stumps.
13. Miscellaneous: shoes, fence posts, sea shells, crab holes, leaf axils and engine parts.

Other observations: In addition to examination of human subjects for filariasis and mosquito surveys, the bloods of a total of 220 animals and birds were examined from the ten experimental areas on Tahiti and Maiao. Animals examined included 46 dogs, 24 cats, 2 cows, 7 horses, 2 goats, 15 pigs, 41 chickens, 8 ducks, 43 mynah birds, 9 rats and 6 lizards. Twenty-six dogs and 4 cats, or 56.5 and 12.5 per cent respectively, were positive for microfilariae of *Dirofilaria immitis*. A larval nematode, thought to be a type of filarial larva, was recovered from one pig on Maiao.

Adult filarial parasites were recovered from two subjects in Tahiti during the spring of 1950. Seven males, six females and one immature specimen, all alive, were recovered from a pendulous mass hanging from the inguinal region of the right side of a 45 year old Tahitian male who had resided for 36 years in Lower Paea, area VIII. Also ten live adult worms were recovered at autopsy from a Rarotongan male, estimated 42 years of age who had been a resident of Makatea, French Oceania for 20 years. Several male and female filariae from each of the subjects were carefully studied and conformed to the anatomical description of *Wuchereria bancrofti* (Cobbold).

In addition, the microfilariae of a large series of carefully prepared smears from numerous subjects, particularly those persons utilized in periodicity studies (Edgar *et al.*), were studied and compared with specimens of *Wuchereria bancrofti* from Okinawa, Saipan, Puerto Rico and India, and there appeared to be no morphologic differences between the microfilariae of Tahiti and those from the above mentioned regions.

Effect of diethylcarbamazine on microfilariae: The results of the several methods of control on the percentage of persons with microfilariae before, and at 6 and 12 months after, control measures which were begun in areas I to VII are shown in Table 4. As expected, in areas I, IV, and VI where no treatment was used, no perceptible reduction occurred during the year. The combined percentages of positives before application of control measures, and at 6 and 12 months, for these three groups where treatment was not given, were 41.0, 43.3 and 44.2 respectively. However, a noticeable drop occurred in the percentage of positives in each of the four areas where diethylcarbamazine was administered. The

percentage positives for all four treatment areas before the application of control measures and at 6 and 12 months, were 34.4, 21.5 and 19.6 respectively, and most of the positives had very low microfilarial counts after treatment. It should be mentioned that included in Table 4 are two untreated negative persons in area VII who became positive during the experiment. In area III, one positive infant did not receive treatment. Also, not shown in Table 4, are the results of migrations of people who moved into the treated areas during the course of the experiment. However, an analysis of data for all subjects examined at 6 and 12 months revealed that the immigrants increased the percentage of positives by only 1.0 per cent. The data presented in Table 4 for areas where subjects received diethylcarbamazine are in close agreement with the findings in Upper Paea and Maiao, i.e., Areas IX and X.

TABLE 4

Number and percentage of persons positive for microfilariae in experimental areas I to VII before and during treatment with diethylcarbamazine. Tahiti, 1949–50

AREA NUMBER	BEFORE*			6 MONTHS			12 MONTHS		
	No. Exam.	Positive		No. Exam.	Positive		No. Exam.	Positive	
		No.	Per cent		No.	Per cent		No.	Per cent
I	55	21	38.2	55	22	40.0	53	23	43.4
II	53	16	30.2	53	11	20.8	46	9	19.6
III	34	14	41.2	34	9	26.5	31	6	19.4
IV	45	13	28.9	45	15	33.3	31	9	29.0
V	57	18	31.6	57	8	14.0	52	7	13.5
VI	34	21	61.8	34	21	61.8	29	18	62.1
VII	51	19	37.3	51	14	27.5	39	11	28.2
Areas treated	195	67	34.4	195	42	21.5	168	33	19.6
Areas not treated	134	55	41.0	134	58	43.3	113	50	44.2

* Only those persons who were examined before and at six months are included; a few of these same persons were missed at twelve months.

A comparison of the percentage of persons positive before and after treatment, however, does not show the effect of the drug so clearly as does a comparison of the actual numbers of microfilariae per 20 cmm. of blood per person examined, before and at 6 and 12 months after administration. These data are shown in Table 5.

At 12 months after treatment, the average number of microfilariae per person examined was less than 10 per cent of the original number. The average microfilarial counts for 6 and 12 months post-treatment included counts of seven persons who were positive and not treated or who became positive during the experiment. Not included in Table 5 are the microfilarial counts of positive immigrants who moved to the area during the experiment. When these are included the average count for all persons in areas II, III, V and VII is increased

by 2.6 microfilariae. To illustrate the actual reduction of microfilariae following therapy, the average counts for 45 positive persons who were examined on all four occasions, before treatment, at 3, 6 and 12 months, are shown graphically in Figure 2. Twenty-two of these persons were still negative at 12 months and the average microfilarial count at 12 months was 6.9 per cent of the average count before treatment.

The effect of diethylcarbamazine on clinical filariasis: A careful record was kept of the number of attacks of lymphangitis experienced by all persons in areas I to VII during one year and the results are summarized in Table 6.

It is of interest to note that in both the untreated and treated group only one half of the persons giving a history of lymphangitis prior to the study experienced an attack during one year of observation. Treated persons with a positive

TABLE 5

Average number of microfilariae per person before and during measures in experimental areas. Tahiti and Maiao, 1949–1950

AREA NUMBER	BEFORE		6 MONTHS		12 MONTHS	
	No. exam.	Average No. M/F	No. exam.	Average No. M/F	No. exam.	Average No. M/F
I	55	50.9	55	46.9	47	47.5
II	52	32.0	52	3.3	46	3.9
III	34	56.0	34	1.7	31	1.6
IV	45	47.6	45	32.6	31	34.7
V	57	47.8	57	1.5	48	0.5
VI	31	106.2	31	115.7	27	112.6
VII	45	29.0	45	2.5	36	3.3
VIII	302	37.0	302	37.0	discontinued	
IX	422	15.7*	—	—	322	3.1†
X	169	34.2	—	—	155	1.4‡

* Average affected by treatment of 51 cases with diethylcarbamazine in March 1948.
† Eleven to 12 months post-treatment.
‡ Ten months post-treatment.

history would be expected to have approximately the same frequency of attacks as untreated persons if diethylcarbamazine in this dosage was ineffective. The expected number of attacks to be observed in the treated group is 60. The actual figure is 52. The expected number of attacks to be observed in the treated group without a previous history of lymphangitis is three, while the observed number is 7. The frequency of attacks in each experimental area showed no change with the type of program.

Tahitians often state that attacks of lymphangitis are more common during a certain season of the year, but data gathered in this study failed to reveal any evidence of a seasonal variation.

The effect of treatment on other manifestations of clinical filariasis was also of interest. The physical examination made at 16 months post-treatment in Upper Paea, area IX, revealed that 70 treated persons still had essentially the

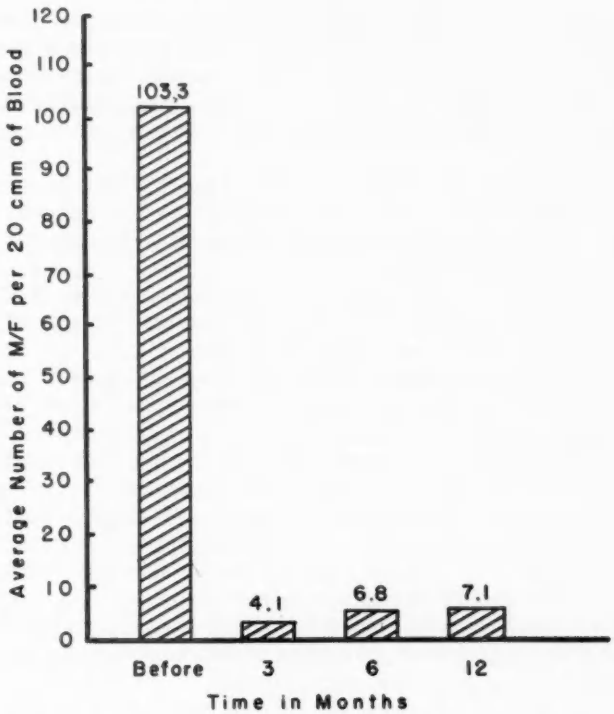


FIG. 2. Average number of microfilariae in 20 cmm. of blood per person before and after diethylcarbamazine therapy. Tahiti, 1949–50.

TABLE 6

Number of attacks of lymphangitis observed among treated and untreated Tahitians classified according to presence or absence of a previous positive history, and the presence or absence of attacks during the post-treatment period. Experimental areas, Tahiti, 1949–1950*

GROUPS OF TAHITIANS OBSERVED POST-TREATMENT		TREATED PERSONS		UNTREATED PERSONS	
		Hist. of attacks previous to treatment		Hist. of attacks previous to treatment	
		Yes	No	Yes	No
Tahitians with no attacks	Person months	186	589	220	2,577
	Number of persons	15	49	18	213
With attacks in post-treatment period	Person months	198	60	223	48
	Number of persons	17	5	19	4
	Number of attacks	52	7	69	11

* With diethylcarbamazine.

same signs they had before treatment with diethylcarbamazine. The right leg of one person was slightly enlarged for the first time during the post-treatment period. This particular subject had an original microfilarial count of 726 per 20 cmm. and after the course of treatment, he experienced frequent attacks of lymphangitis in his right leg associated on two occasions with abscesses in the right popliteal area.

Similar observations were made concerning persons treated in areas II, III, V, and VII. There was no evidence that the drug caused enlargement or reduction of the epitrochlear nodes, nor was there any evidence that the drug had any effect on the elephantoid organs.

Other pathologic conditions were noted by the senior author in the experimental areas but there was no evidence at all that any other disease was particularly associated with or predisposing to filariasis.

Reactions to diethylcarbamazine: In general, the drug was well tolerated by most subjects, but there were some reactions worth noting. During the course of treatment and in the early post-treatment period, two types of clinical reaction were observed. The first type appeared within the first day after starting medication, and persisted through the 3rd or 4th day in the majority of persons. Fever was the most consistent reaction and was associated with a variety of other transitory symptoms such as fatigue, headache, anorexia, pruritus, joint pains, pains in the abdomen and femoral area, and nausea. Reactions appeared to be much more severe in older persons than in children.

The second type of reaction appeared 3 to 15 days after the systemic reaction and occurred in 59 per cent of the persons exhibiting clinical filariasis while in the asymptomatic carriers it was present in only 33 per cent. These reactions consisted of pruritus, enlarged and painful lymph nodes which suppurated in 3 per cent of the cases, scrotal enlargement with tenderness, and skin eruptions ranging from urticaria and small vesicular rashes to formation of bullae. Abscesses developed in 5 per cent of the treated persons during the first six weeks. Bullous eruptions over the arm and back occurred among 3 per cent within the first month.

Laboratory analyses were performed on some of the patients who received treatment in areas IX and X. Total white cell counts were obtained on 32 persons before treatment, on the 1st, 2nd, 3rd and 7th days of treatment, 1–2 months after treatment, and at 16 months after treatment. A slight leucocytosis was observed during the course of treatment. Total white counts after treatment did not vary significantly from the white cell counts before treatment. Specimens of urine were collected for analysis from 54 persons before the administration of the drug, and on the 1st, 2nd, 3rd and 7th days during the course of treatment, at periodic intervals thereafter, and at 16 months after treatment; of these, 48 showed no albumin. The only significant change during the course of treatment was the appearance of albumin in 25 of the 48 persons who were originally negative. In these twenty-five, the albuminuria was transitory as judged by subsequent tests. The other 23 in which albumin was absent before treatment, likewise showed no albumin after treatment. Stools

were examined for worms before and after treatment from 50 individuals in area IX and for 24 in area X and it was observed that many ascarids were eliminated during the course of the treatment. The percentage of persons still positive at the close of the treatment was high, however, and it was recognized that the required dosage of diethylcarbamazine as an ascaricide had still to be determined.

MOSQUITO POPULATIONS

Because of the many variables which may occur in different areas selected for observation with reference to:

(1) density of human or animal populations,
(2) proximity of collection area to bush or cleared spaces,
(3) habits and flight range of mosquitoes,
(4) fluctuation of climatic factors such as temperature, humidity, wind and rainfall,
(5) difference in personal factors in attracting and collecting mosquitoes,

any attempt to establish indices by which to evaluate the exact rise and fall of a mosquito population is extremely difficult.

In this investigation, four studies were undertaken in each area to obtain possible indices by which the progress of the mosquito control program might be evaluated. These were:

(1) periodic collection of mosquito larvae,
(2) periodic collection of adult mosquitoes at resting stations,
(3) periodic collection of adult mosquitoes at biting stations,
(4) dissection of the mosquitoes, collected in each area, to determine the presence of infective stages of filaria larvae.

Collection of larvae

Larva surveys were conducted in all areas except X at monthly intervals during the period of study. All household premises were surveyed and the search was extended to a radius of 75 yards around each area. Before control measures were instituted mosquito breeding was found to be widespread in all areas and occurred in most of the types of containers listed earlier in this paper. No reduction in breeding was observed in areas I, II, and III in which no mosquito control measures were employed. However, after "clean-up" or sanitation was enforced in areas IV and V, DDT applied to areas VI and VII and both sanitation and DDT applied to areas VIII and IX, mosquito breeding was found to be confined almost exclusively to rat-eaten coconuts, tree holes and swampy places. The immediate grounds of all of the homes in these six areas were essentially free of breeding sites.

Collection of adult mosquitoes

Twice monthly adult mosquito surveys were made at three resting stations and at three biting stations in areas I to VII throughout the whole 14 months of the study. The collections during July and August, 1949, were made prior to

the institution of experimental measures. Collections thereafter were made during the experiment. Results of these collections are tabulated in Table 7. It will be observed that the average number of mosquitoes taken at each collection before the experiment began in July and August, 1949, is compared, first with the same two month period for 1950, and second with the monthly average for the whole year.

Again, it will be observed that there was little or no decrease in the adult mosquito population in those areas where neither sanitation nor DDT was used, the one exception being area III. In those areas where sanitation alone was used, where DDT alone was used or where both measures were employed at the same time, reductions were observed on the whole, these being more apparent in the number of *C. quinquefasciatus* at the resting stations than of

TABLE 7

Average number of adult mosquitoes at resting and at biting stations in experimental areas for July and August, 1949 and 1950, and monthly average from September 1, 1949 to September 1, 1950

AREAS	RESTING STATIONS*			BITING STATIONS		
	Before Exp., i.e. July and August 1949	July and August 1950	Monthly ave. September 1949–50	Before Exp., i.e. July and Aug. 49	July and August 1950	Monthly ave. Sept. 1949–50
I	4	2	2	10	8	18
II	4	5	9	10	23	36
III	19	5	9	101	41	54
IV	35	0	8	43	22	17
V	16	3	3	46	32	48
VI	4	1	1	12	3	15
VII	19	1	1	69	41	33
VIII	†No Observ.	0.14	0	No Observ.	0	1

* Most mosquitoes at resting stations were *Culex quinquefasciatus* but at biting stations over 99% were *Aedes pseudoscutellaris*.

† Although no pre-control counts were made, it was easy to find adult mosquitoes prior to control measures.

A. pseudoscutellaris at the biting stations. It would thus appear from the data in Table 7 that the application of sanitation alone or of DDT alone, as used in this study, is less successful in reducing *Aedes pseudoscutellaris* than *Culex quinquefasciatus*.

Infective filarial larvae in dissected mosquitoes

Since *A. pseudoscutellaris* appears to be the most important vector of filariasis in Tahiti and data regarding *C. quinquefasciatus* are few, only the results of dissections of *A. pseudoscutellaris* will be reviewed at this time. Table 8 shows the infective larvae recovered from this species before the experiment, a year later and also, for comparison, the average of four collections made at three month intervals during the whole year.

Allowing for the explanations concerning the data at the bottom of Table 8, one may make the following observations from this table:

(1) The percentage of mosquitoes with infective larvae in area I, which was the untouched control area, exhibited no significant reduction throughout the period of the experiment.

(2) In areas II to X, i.e. where experimental measures were employed, reductions in the percentages of mosquitoes with infective larvae were apparent. This is observable both for the averages for the year and for the collection in

TABLE 8

Aedes pseudoscutellaris *dissected from experimental areas showing percentages with infective stage of filarial larvae*

AREA	BEFORE EXPERIMENT AUGUST 1949			AT CLOSE OF EXPERIMENT SEPTEMBER 1950			TOTAL FOR 3, 6, 9, AND 12 MONTHS		
	No. dissected	No. with larvae	% with larvae	No. dissected	No. with larvae	% with larvae	No. dissected	No. with larvae	% with larvae
I	106[1]	5	4.7	22	2	9.1	138	6	4.4
II	61	+[2]	?	73	1	1.4	182	1	0.5
III	80	8	10.0	97	1	1.0[3]	308	2	0.6
VI	51	3	5.9	97	0	0.0	299	0	0.0
V	64	3	4.7	99	0	0.0	346	5	1.4
VI	62	4	6.5	99	2	2.0	309	7	2.3
VII	56	4	7.1	96	2	2.1	271	4	1.5
VIII	26	3	11.5	22	1	4.0	202	4[4]	2.0
IX	Not done	—	—	41	1	2.4	262	6[4]	2.3
X	88	3	3.4	113	1	0.9	230	2	0.9

[1] Dissections of 48 were made in March 1949.

[2] Protocols of 25 dissections, among which were known positives, were lost. Therefore the exact number cannot be stated.

[3] This one positive specimen contained 10 infective stage larvae and it was taken near a house into which immigrants to the area had moved. One of these immigrants showed 101 microfilariae per 20 cmm. of blood.

[4] A majority of the mosquitoes from areas VIII and IX that were dissected during the control period were selected only from or near homes known to have infective persons; whereas mosquitoes from other areas came from near all homes regardless of the presence or absence of carriers.

September, 1950. This conclusion does not apply to area IX, where pretreatment surveys were not made.

The data collected in this study are too few to warrant an attempt to draw statistically significant conclusions regarding the superiority of any single method employed in this study, and they are reported with the realization that they merely exhibit trends which may or may not be confirmed by future studies. Nor is it possible to answer the question at present as to whether any one of the four above mentioned indices can be used to advantage in evaluating the progress of a filariasis control program. Counts of mosquito breeding places or of mosquito larvae and counts of adult mosquitoes collected at biting stations or at resting stations, may be used to provide information concerning the general

effectiveness of a mosquito control program. It is extremely difficult, however, to apply these indices to quantitative analysis because of the many variables mentioned above. Additional information will have to be procured before it is possible to state which one of these methods is most applicable to the program in Tahiti.

The infective filaria larval index in mosquitoes has merit as a means of evaluating the effectiveness of a mosquito control program, providing the collection of mosquitoes can be standardized. The many variable factors, such as the proximity of collecting stations to infected people, the microfilarial index of the infected people, factors involving the bionomics of the mosquitoes, and seasonal and climatic variations all require evaluation. Samples of mosquitoes from a given area, to be of statistical significance, should be sufficiently numerous to outweigh the above mentioned variables. It is obvious that to apply this procedure in an accurate quantitative way would be time consuming and expensive. For this reason, additional study is required before specific recommendations can be made for its use either in this program or in general.

Other observations

The total rainfall during the 12 months' experiment in areas I to VII, was 51.5, 141.4, 132.3, 104.2, 132.3, 51.5 and 92.7 inches respectively as compared with 82.7, 145.1, 145.1, 145.1, 145.1, 82.7 and 78.8 inches respectively for the areas or nearby stations for the previous year. Thus it is to be noted that there was considerable difference in the amount of rainfall in the areas. Areas VI and VII had about half the rainfall of areas II to V. The effect of the lesser rainfall in areas I, VI and VII was reflected in the larva and adult mosquito surveys.

An analysis of the data on migration revealed that there was considerable movement of people in and out of the areas with an average exposure of inhabitants to other persons 22 per cent of the time, with the greatest number of visitors in area VII and the fewest in area III. A total of 10 infected *A. pseudoscutellaris* collected before and during this study from homes not having carrier inhabitants suggests the probability that they had acquired their infective meal while feeding on visitors who were carriers.

A survey of individuals sleeping under mosquito nets during the greater parts of their lives yielded too few data to warrant any conclusion as to the effectiveness of mosquito nets in preventing infection.

In a combined research and control program of the type here reviewed, changes both in methods of evaluation and in application of procedures may be required from time to time.

The present report is an attempt to evaluate the program in its first phase by:—

(1) recording procedures of making surveys and reporting results,
(2) evaluating the clinical aspects of filariasis,
(3) comparing the effect of two main types of filariasis control,
    (a) drug administration in the form of diethylcarbamazine directed toward the destruction of microfilariae and
    (b) mosquito control.

Attempts to evaluate a program to control filariasis must necessarily extend over a long period of time because the disease exhibits so many manifestations in man, ranging from mild attacks of lymphangitis with or without fever to chronic and debilitating elephantiasis which may take years to develop. It is not possible as yet to say to what extent the success of a program can be judged by:

(1) making periodic counts of microfilariae in the treated segment of the population,
(2) evaluating a mosquito control program or
(3) determining the infective filaria larval index in a mosquito population at regular intervals.

Each of these three indices must necessarily be considered as preliminary in nature, the final evaluation of success being the reduction in clinical manifestations in a given population. This will require years to evaluate and likewise require carefully collected clinical observations made at the beginning of a program for base line comparisons. It is this material for base line comparisons which has been collected with care and which will be available for comparison with future studies.

Although the incidence of microfilariae in carriers was reduced in all areas for periods of one year or more where treatment was administered, the importance of low density carriers in the transmission of filariasis is not completely understood. It is possible to obtain laboratory infections with *A. pseudoscutellaris* fed on subjects with counts of less than 5 microfilariae per 20 cmm. of blood. Also, whether mosquitoes with a few infective larvae are more important vectors in nature than mosquitoes with many larvae is as yet not known. The future direction of control measures must be guided by experimental findings on these and other topics. However, it was observed in this study that the percentage of mosquitoes harboring infective filaria larvae was reduced significantly in areas where treatment was administered.

SUMMARY

A survey of 10 experimental areas on the islands of Tahiti and Maiao revealed an incidence of microfilarial carriers of 33% in people over 5 years of age. Males had an average microfilarial density in capillary blood approximately twice that of females. The microfilarial density increased with age to a peak in the 40–49 year age group. Forty-one per cent of the persons examined over one year of age had clinical filariasis, the first attack occurring most often in the age period 20–29 years. Adult specimens of filaria were recovered from two individuals and both these and the microfilariae resembled descriptions of *Wuchereria bancrofti* from other regions.

Nine species of mosquito were found, the most common being the day biting *Aedes pseudoscutellaris* (the principal vector), and the night biting *Culex quinquefasciatus*.

Diethylcarbamazine (Hetrazan), at a dosage of 2 mg./kg. T.I.D. for 7 days, reduced the microfilarial incidence from 34.4 per cent before treatment to 19.6 per cent at the end of a year and the microfilarial density of the remaining treated

positive subjects was less than 10 per cent of the original concentration. There was no reduction in untreated comparison areas. Treatment, however, did not reduce the frequency or severity of clinical filariasis. No seasonal variation in the frequency of attacks of lymphangitis was observed. The drug was well tolerated, although more than half the subjects showed transitory reactions. The treatment campaign appeared to have no effect on the mosquito population, nor did DDT and sanitation, in the course of one year, have an effect upon microfilarial rates or densities, although the evidence suggests that DDT and sanitation reduced the adult mosquito population. No evidence of mosquito resistance to the toxicant was observed during one year.

In the highly endemic area, a combination of treatment, sanitation and use of insecticides would seem to offer the best likelihood of success in the control of filariasis, and studies are being continued in Tahiti to determine the most effective mode of application and association of these measures.

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RESUME

1. Une enquête portant sur la population de 10 régions expérimentales dans les îles de Tahiti et Maiao a révélé une forte proportion de filariose de Bancroft parmi une population de 1265 personnes examinées. Le nombre de porteurs de microfilaires à partir de l'âge de cinq ans était 407 soit 33.17%.

2. Quarante pour cent des hommes dans les régions 1 à 7 et 33% des femmes etaient porteurs de microfilaires, La densité moyenne des microfilaires par 20 mm^3 de sang chez 171 hommes était approximativement deux fois plus élevée que chez 115 femmes, soit respectivement 130,4 et 66,7.

3. Le chiffre moyen de microfilaires par 20 mm^3 de sang chez les individus positifs était 127,7 et augmentait avec l'âge pour atteindre un maximum de 268 entre 40 et 49 ans. Le nombre le plus élevé trouvé a été 1094 microfilaires chez un homme de 42 ans et l'enfant le plus jeune trouvé positif fut une fille de 14 mois.

4. 121 ou 32% des 376 personnes au-dessus d'un an dans les regions 1 à 7, alors que 77 ou 46,7% des 165 personnes au-dessus de 3 ans à Maiao présen-

taient des signes cliniques. Dans un groupe de 85 personnes avec lymphangite, le nombre le plus élevé, 30 ou 35,3% ont présenté leur première attaque de lymphangite entre 20 et 29 ans.

5. Neuf espèces de moustiques ont été rencontrées dans les régions experimentales au cours de l'étude entreprise. Les deux espèces les plus communes sont le moustique piquant le jour, *Aedes pseudoscutellaris* qui est le vecteur principal, et le moustique piquant la nuit, *Culex quinquefasciatus*.

6. Les lieux de reproduction principaux sont les noix de coco mangées par les rats, les ornières et les marécages, les pirogues, les trous d'arbres, les boîtes de conserve et d'autres récipients moins importants.

7. 220 animaux furent examinés pour rechercher les microfilaires possibles; 26 ou 56,5% des 46 chiens et 4 ou 12,5% des 24 chats ont été trouvés porteurs de microfilaires de *Dirofilaria immitis*.

8. Des spécimens de filaires adultes furent trouvés chez deux sujets, l'un habitant Makatéa et l'autre la région 7. Le parasite ne parait pas differer morphologiquement de *Wuchereria bancrofti* (Cobbold).

9. Les microfilaires des habitants de Tahiti ou d'autres îles d'Océanie Francaise, ont de même été trouvées indifferentiables des microfilaires de *W. bancrofti* provenant de plusieurs autres territoires.

10. Les régions oú l'Hétrazan fut administré oralement à raison de 2 mmg. par kilogramme de poids, trois fois par jour, pendant sept jours, et où 34% des habitants étaient porteurs de microfilaires, ont montré une réduction de ce pourcentage à 19,4% à la fin de l'année.

Dans les régions ou l'Hétrazan n'était pas administré aucune réduction n'est apparue.

11. Il n'existe pas de réelle preuve dans cette étude que l'Hétrazan réduise la fréquence ou la séverité des signes et symptômes de filariose. De même qu'il n'y a pas de preuve que les attaques de lymphangite soient plus fréquentes pendant certaines saisons de l'année.

12. l'Hétrazan, ainsi administré, a été bien toleré par la plupart des patients. Cependant, deux types de réactions transitoires furent observés. Le 1er groupe de réaction survient entre le 1er et 3ème ou 4ème jours de traitement, les symptômes les plus communs étant la fièvre, souvent accompagnée de fatigue, céphalée, anorexie, prurit, douleurs abdominales, nausée, arthralgies. Le second groupe de réactions survient entre 3 et 15 jours et consistent en prurit, adénopathies douloureuses, urticaire et éruption bulleuse.

13. Appliquée pendant une année l'administration d'Hétrazan n'apparait pas avoir d'effet sur la population des moustiques. Aussi les mesures de contrôle du moustique, DDT et assainissement n'avaient pas d'effet sur la fréquence ou la densité des microfilaires.

14. Il est prouvé que le DDT réduit la population des moustiques.

15. Dans des régions de haute endémicité, l'administration d'Hétrazan à toute la population combinée avec le contrôle des moustiques par assainissement et emploi judicieux du DDT est la méthode la plus sure de contrôle de la filariose. Cependant il n'est pas toujours possible d'employer toutes les mesures

ensemble et c'est le but des études en cours a Tahiti, de determiner les combinaisons possibles et efficaces qui peuvent être appliquées.

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FILARIASIS IN AMERICAN SAMOA

II. Evidence of Transmission outside of Villages

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Studies on filariasis in American Samoa were undertaken in 1948 by the Bureau of Medicine and Surgery, United States Navy, and the Naval Medical Research Institute with the cooperation of the Government of American Samoa. The basic problem was to evaluate and develop methods for the control of this disease, which is caused by aperiodic *Wuchereria bancrofti*. These parasites are most vulnerable to attack either as microfilariae in the peripheral blood of man or as larvae in the mosquito vectors. Antifilarial drugs can be used to interrupt the life cycle of the worms in man and mosquito control measures to interrupt the cycle in the vectors, but, in order to apply the mosquito control measures most effectively, it is essential to know where transmission occurs.

Byrd, St. Amant, and Bromberg (1945) regarded the village as a "hyperendemic focus of infection". This concept has been widely accepted. However, in reviewing the known details of the epidemiology of filariasis, transmission elsewhere is also suggested. As originally described by Bahr (1912) in Fiji and confirmed by others including ourselves in Samoa, the microfilariae of aperiodic *W. bancrofti* are present in the peripheral blood both day and night. However, transmission is limited to the daylight hours because the only important vector in Samoa is *Aedes pseudoscutellaris*, a widely distributed mosquito with diurnal habits, feeding by preference during the cooler hours after daybreak and before sunset rather than in the midday heat. Furthermore, the Samoans prefer these hours for those activities which take them outside of the villages. It is not surprising then that transmission along a trail outside of the native village was demonstrated by Byrd and his coworkers despite their conclusion quoted above. It is the purpose of this paper to present additional evidence that filarial infections can be acquired in the "bush", outside of the native villages.

For an understanding of the epidemiology of this disease, it is necessary to describe briefly a typical Samoan village and to define the area included therein. Most of the villages follow the same basic plan. They are usually located on the coast or along the main road. Between the beach or the road and the houses is a *malae* or village green. The *malae*, with only a few scattered coconut and breadfruit trees, is cleared of undergrowth and is covered only by closely cropped grass, bare sand or crushed coral. The native houses consist of a single room carpeted with washed coral pebbles and covered by a high thatched roof which

[1] The opinions and statements expressed are those of the authors and not necessarily those of the Navy Department.

These researches were conducted with the technical assistance of Carlos Schultz, HMC, USN and Donald Rankin, HMC, USN.

is supported by central and marginal posts. There are no permanent side walls, but in inclement weather woven blinds are lowered. Cooking is done in separate sheds located varying distances to the rear of the houses. The bush, which usually begins immediately behind the cook sheds, may be either jungle or coconut and banana groves with luxuriant undergrowth. Most of the houses in the Samoan villages are thus in a clearing but some of the houses, particularly those in the small villages in compressed coves, may be actually within the bush. Well worn trails lead off through the bush to the plantations and to other villages. Our concept of a village is the area from the beach or road to the cook sheds behind the houses.

METHODS

Ten villages were included in these studies: nine on the island of Tutuila and one on the adjacent small island of Aunuu. Four mosquito collecting stations were established for each village (a) on the open *malae* near the center of the village and approximately 25 yards from the nearest house; (b) in one of the houses selected at random; (c) on a trail in the nearby bush 50 to 100 yards from the houses; and (d) further along a trail in the bush usually in banana or coconut plantations 150 to 250 yards from the houses. Selection of these sites depended primarily upon terrain, native cooperation, and the proximity of other villages. All collections associated with a single village were made within a period of 90 minutes on a single afternoon by two of the five men in the research unit. On arrival at a collecting station the collectors spent a minute or two unpacking the equipment, then, using aspirator-type collecting tubes, they endeavored to collect all mosquitoes attempting to feed on them in a period of ten minutes. Sometimes a curious Samoan child or two provided additional bait especially on the *malaes* and in the houses. This was unavoidable and occurred sporadically in all of the villages. Over 1700 mosquitoes were collected during scheduled periodic surveys. These mosquitoes together with 649 obtained on special surveys were examined for larvae of *W. bancrofti*. Only those insects with developing or infective larvae in the tissues were considered infected.[2] The comparatively few larvae of *Dirofilaria immitis* (of the dog) which were found, are not of course, included with these data.

RESULTS

A summary of the collections from the ten villages indicates that *A. pseudoscutellaris* is primarily a bush mosquito, for it was most abundant at the stations located at the greatest distance from the village and least abundant on the open *malaes* (Fig. 1). Furthermore, the extent to which the mosquitoes prefer the bush over the artificial shelter of the houses is partially masked by the method of averaging the data from all the villages. The location of the houses in the villages follows two distinct patterns. In the four small villages located in little coves, the underbrush of the steep mountainsides encroached on the houses,

[2] Thus the two mosquitoes found with only freshly ingested microfilariae in the stomach are not included as infected mosquitoes.

while in the other six larger villages the land was flatter and the houses were separated from the bush by cleared areas 25 to 50 yards wide. When the data from the two types of villages are separated, significant differences appear in the numbers of mosquitoes collected in the houses. Many more mosquitoes were collected in the houses near or in the bush than in those with extensive cleared

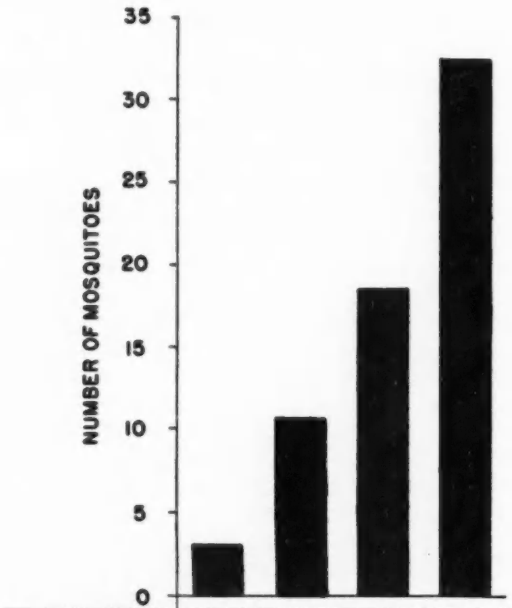

FIG. 1. SUMMARY OF THE DENSITIES OF AVERAGE NUMBER PER SURVEY OF AEDES PSEUDOSCUTELLARIS (TEN MINUTE COLLECTIONS FROM HUMAN BAIT) FROM TEN SAMOAN VILLAGES.

FIG. 1

areas around them (Fig. 2). The similarities should be noted in the collections in the houses close to the undergrowth and those in the nearby bush as well as in the collections in the bush in both types of villages.

The incidence of infection in mosquitoes does not show the well marked distribution pattern with the village as the hyperendemic focus described by

Byrd and his coworkers. The percentage of infected mosquitoes for all villages was 6.9 on the *malae*, 5.2 in the houses, 2.9 in the nearby bush, and 4.9 in the deeper bush. Separation of the infections according to the types of village again emphasizes the very minor role of the houses of the open villages in the transmission of filariasis (Fig. 3).

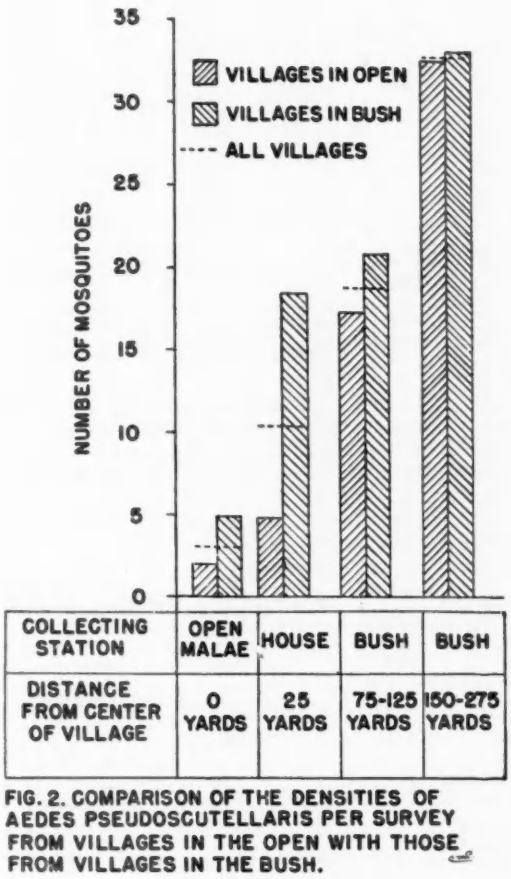

FIG. 2. COMPARISON OF THE DENSITIES OF AEDES PSEUDOSCUTELLARIS PER SURVEY FROM VILLAGES IN THE OPEN WITH THOSE FROM VILLAGES IN THE BUSH.

FIG. 2

Even in the bush, collections did not always yield infected mosquitoes. The frequency of infected mosquitoes varied and appeared to be related to the amount of native traffic. In addition to collections at these specified sites around the villages where Samoans frequently come in contact with the mosquitoes, random collections were made in various other parts of the island. There was

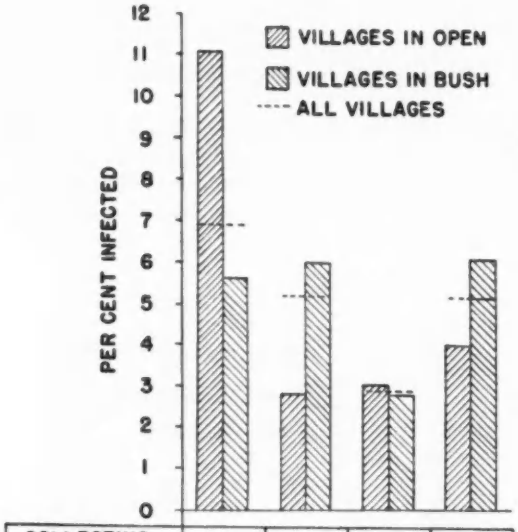

FIG. 3. COMPARISON OF THE INFECTION RATES IN AEDES PSEUDOSCUTELLARIS FROM VILLAGES IN THE OPEN WITH THOSE FROM VILLAGES IN THE BUSH.

FIG. 3

TABLE 1

Summary tabulation of mosquito (A. pseudoscutellaris) *densities (10 minute collections) and mosquito infections with nonperiodic* W. bancrofti

Collecting Station	MALAE			HOUSE			BUSH 50–100 YDS.			BUSH 150–250 YDS.		
Type of village	all	open	bush*	all	open	bush*	all	open	bush*	all	open	bush*
Density												
No. of surveys	27	15	12	27	15	12	59	35	24	8	5	3
No. of mosquitoes collected	83	27	56	292	71	221	1098	600	498	261	162	99
No. of mosquitoes per survey	3.1	1.8	4.8	10.8	4.7	18.4	18.6	17.1	20.7	32.6	32.4	33.0
Infection												
Number dissected	81	27	54	289	71	218	1766†	1098†	668†	247	148	99
Number infected	6	3	3	15	2	13	52	33	19	12	6	6
% infected	6.9	11.1	5.6	5.2	2.8	6.0	2.9	3.0	2.8	4.9	4.1	6.1
Index of transmission												
	0.2	0.2	0.3	0.6	0.1	1.1	0.5	0.5	0.6	1.6	1.3	2.0

* Small villages in which the bush encloses many houses.

† Includes dissection records from special surveys.

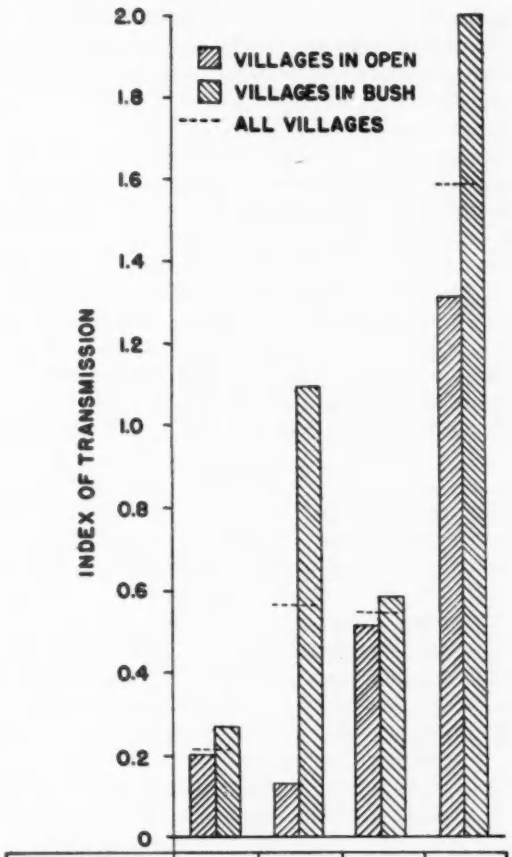

FIG.4. COMPARISON OF INDICES OF TRANSMISSION FOR VILLAGES IN THE OPEN WITH THOSE FOR VILLAGES IN THE BUSH.

FIG. 4

no place in the jungle or bush free of these mosquitoes. However, infected mosquitoes were never found in those areas where there was no evidence of recent visits by man.

Although the percentage of mosquitoes infected has frequently been used to indicate transmission, the results are often misleading because such data do not

take into consideration the density of mosquitoes in the various areas. Thus, because of a low mosquito density, the 11.1 per cent infection from the *malaes* of the open villages is based on the examination of only a few mosquitoes whereas the 3.0 per cent infection from stations 75 to 150 yards in the bush at the same villages represents many more (Table 1). Therefore, by themselves, these percentages tell us little. In determining transmission possibilities the numbers of mosquitoes involved must also be considered. An index of transmission can be calculated by multiplying the density (the average number of mosquitoes per survey) by the proportion of mosquitoes infected. Application of this index to the data already presented radically alters the interpretation of the importance of the various areas in transmission (Fig. 4). Mosquitoes on the open *malaes* of all villages and in houses in cleared areas (open villages) are of little importance in transmission of filariasis as compared with the mosquitoes in the nearby bush and in houses close to the bush. The index of transmission is extremely high for the mosquitoes collected in the bush 175 to 275 yards from the center of all villages, exceeding the combined indices of the other three collection stations.

DISCUSSION

These mosquito indices depict the areas of potential transmission. They are in sharp contrast to the generally accepted theories. These data demonstrate that *A. pseudoscutellaris* is found much more plentifully associated with wild or natural cover than with artificial or domestic cover provided by human habitations. Thus, in American Samoa at least, transmission of filariasis occurs in a "wild ecological niche". Since the mosquitoes feed exclusively in the daytime, it would appear that a single hour during the day spent in the bush, along the trail, or in the plantation, offers greater opportunity for infection than the remainder of the day and night spent in the typical open village. Only in the small villages, in constricted coves in which the bush encloses many of the houses, does the index of transmission suggest any appreciable domestic transmission. This, in itself, further emphasizes the association of transmission with natural cover.

It may be noted that data on the age and sex distribution of microfilaremia in the native Samoans, on the habits of the Samoan people and on the biting habits of this mosquito, which will be published in detail later, all support the concept that this infection, in the area studied, is primarily transmitted in the wild.

Byrd, St. Amant, and Bromberg (1945) also emphasized that *A. pseudoscutellaris* is a "bush mosquito" and showed that the mere passing of Samoans twice daily was sufficient to produce 13 per cent infection in these mosquitoes collected along the trail 300 yards from the nearest village. These workers also presented evidence that the flight range of this species is, in general, somewhat less than 100 yards. However, they were led to conclude that "the native village is considered a hyperendemic focus of infection". This conclusion, the full implication of which we cannot support and which seems to us to be in conflict with most

of their data, is supported only by a higher percentage of infection in the village mosquitoes than in the bush mosquitoes. No data are given on the density of the mosquitoes in the particular villages studied or the nature of the environment, although one village is reported to be "completely surrounded by dense jungle". Furthermore, no information is given on the sites in the bush at which mosquitoes were collected. The infection in the jungle mosquitoes is definitely spotty in its distribution, but both our data and those of Byrd and his coworkers demonstrate that the infection can be high where there is only nominal amount of contact with the Samoans. The village is the focus of infection only in the sense that it is the villagers who are the sources of infection for mosquitoes.

O'Conner (1932) has presented evidence that such contact with the bush or plantation is alone sufficient to maintain the endemicity of the disease. His study was made in the Ellice Islands where the parasite and the vector are the same as in Samoa. On Nukulailai atoll, the village was located on an island which was well cleared of bush and on which *Aedes pseudoscutellaris* was rarely seen. However, these mosquitoes were common in the plantations which were on another island across the lagoon. Because of frequent storms, children seldom were taken to the plantations. Examination of the population of the village failed to demonstrate microfilariae in the blood of children under 10 years of age, whereas they were found, on a single examination, in the blood of 23 of the 77 adults (30%). On this atoll the infections must have been acquired in the plantations.

The evidence of nondomestic transmission of filariasis is not confined to the nonperiodic form *W. bancrofti* in Samoa and other Pacific islands. Poynton and Hodgkin (1938) presented data which clearly indicate that in the principal endemic areas of the Federated Malay States, *W. malayi* is primarily transmitted in the plantation areas or along the trails thereto by several species of *Mansonia*. It may be well to completely reexamine the usual concept that *Wuchereria bancrofti* develops an endemic infection as a result of household transmission. Certainly in large areas, where *Culex quinquefasciatus* is the principal vector of the periodic form, the infection appears to be maintained by domestic transmission. However, it is probable that in American Samoa the amount of transmission of the nonperiodic form in the houses, or within the village generally, could not alone maintain the infection. Thus, it is important that the data on transmission of this same form in other Pacific islands be reexamined to determine more clearly the nature of transmission in the several islands. Since *W. malaya* also is maintained, in the Federated Malay States, as a result of transmission in the bush, it would be well to consider this possibility for the periodic form of *W. bancrofti* is those tropical areas where neither *C. quinquefasciatus* not any other highly domestic mosquito is the vector.

Thus with filariasis, as with malaria, an understanding of the epidemiology and the development of control in any given area must focus sharply on the habits and habitat of the principal vector in that area. In American Samoa, residual spray in the houses cannot be expected to effect any measure of control.

The attack on the mosquito must be focused in the bush. Only where the bush encompasses the individual house or encroaches on the village would any attack on the mosquito near the dwellings be of value.

SUMMARY AND CONCLUSIONS

The density of *Aedes pseudoscutellaris* and the percentage of infection with the developing stages of the nonperiodic form of *Wuchereria bancrofti* are combined into an "index of transmission" in different sites. Evidence is presented, on this basis, that transmission, in American Samoa, is primarily in the bush, along the trails and in the plantations. Transmission within the village proper is of little concern. It is suggested that in other Pacific islands, where this same infection and same mosquito vector occur, the possible role of this same type of environment in the transmission of the disease should be considered.

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SCHISTOSOMIASIS AND MOLLUSCACIDES

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INTRODUCTION

The control of schistosomiasis is one of the important problems in modern tropical medicine. An obvious method of combating the disease is to find practical molluscacides to eliminate the snails that serve as intermediate hosts. This approach is of particular interest to the Armed Forces because men operating through an endemic area are very apt to become infected. For this purpose it is important to have a method of control that quickly reduces the intermediate host population to a point where exposure to cercariae is reduced to a minimum. Under these circumstances one does not often have to consider the repopulation of the snail colonies, elimination of reservoir hosts, treatment of local cases in man, etc.

In 1944 and 1945 American troops had their first experience with schistosomiasis japonica in the Leyte campaign. Up to that time copper salts, especially copper sulphate and copper carbonate, were considered the most effective agents for use on aquatic snails. Lime and calcium cyanamide had been rather widely used in Japan against *Oncomelania nosophora*, an amphibious snail. After the experience in the Philippines it seemed advisable to investigate the possibility of finding better molluscacides. The Commission on Schistosomiasis of the Army Epidemiological Board (McMullen *et al.*, 1947); the Naval Medical Research Institute (Stirewalt *et al.*, 1947, 1947a; Kuntz *et al.*, 1950); the Laboratory of Tropical Diseases of the National Institutes of Health (Nolan *et al.*, 1949; Berry *et al.*, 1950); and the 406th Medical General Laboratory (McMullen, 1948, 1949; McMullen *et al.*, 1948, 1951, 1951a; Freytag *et al.*, 1951; Hunter *et al.*, 1951, 1951a) have been the groups primarily interested in this problem. The first and last of these organizations have been particularly active in investigating the activity of chemicals on amphibious snails, the intermediate hosts for *S. japonicum*. The others have done extensive work on the aquatic snails that carry the other two species of human schistosomes.

These efforts have added considerably to the knowledge of molluscacidal agents but it was felt that an extensive investigation of a wide variety of chemicals was in order. In 1950–1951, the Army Medical Research and Development Board approved a project for testing all available chemicals on *O. nosophora*, the intermediate host in Japan. A progress report of the results obtained in 1950 has been prepared (McMullen, 1951). The present paper deals primarily with the results obtained on this project to date. There has not been time to analyze the data completely, but the more obvious results can be made available to others interested in molluscacides.

MATERIALS AND METHODS

The chemical samples were of two types. The American Cyanamid Company prepared mixtures containing approximately 0.5 mgm. of each of 10 chemicals. A total of 413 mixtures was received. They represented practically all of the solids from this collection and the physical nature of the chemicals was the only criterion used in selection. Small samples of separate chemicals were also submitted for testing, listed as follows: Abbott Laboratories (26); American Cyanamid (4); Chemagro Corporation (1); Commercial Solvents (4); Dow Chemical Co. (90); du Pont de Nemours (10); Hercules Powder Co. (148); Julius Hyman Co. (2); Koppers Co. (187); Laboratory of Tropical Diseases (15); Monsanto Chemical Co. (527); Organic Chemistry Department, University of Illinois (624); Pittman-Moore Co. (11); Rohm and Haas Co. (12); R. T. Vanderbilt Co. (12); and miscellaneous chemicals (2). These chemicals had been selected for various reasons, in the hope that they would prove to be molluscacidal.

All of the work was done on *Oncomelania nosophora* from the Yamanashi endemic center. Fresh snails were collected each week-end and the laboratory tests were designed so that they were completed within the following week. The procedure used was a modified plate technique (McMullen, 1949). In early experiments 2 cc. of the various dilutions used were placed on the filter paper in the bottom of the dish. Later it was thought that more liquid would give a better distribution so 1 cc. of solvent was added. The plates were allowed to dry to prevent the toxic action of some of the solvents. When exposure of the snails was started, ten snails were placed in the center of the paper and 3 cc. of water was added. During the first day the plates were examined three times and readings were taken on snail activities. At the same time the snails were centered and water was added as needed. This was repeated once on the second and third days. On the fourth day a reading was taken on activity and then an examination was made to determine the number of dead snails in each plate. A total of 96,270 snails were used in these tests.

Some changes were made in the dilutions used as the experiments progressed. The final technique arrived at with the mixtures was to run two plates at 1:10,000,then if the mixture proved to be active two more plates at the same dilution were made for each of the individual chemicals. Any active chemicals found in these separation tests were followed up with an appropriate series of dilutions.

The final technique adopted for the single chemicals was to use three plates, with dilutions of 1:1,000 to 1:5,000 and 1:10,000. With active compounds additional plates were used as indicated. In addition to the single chemicals, pairs of chemicals were mixed in an attempt to get toxic combinations. Data obtained from the plates were used in calculating the LD_{50} (Reed *et al.*, 1938). This figure is not as exact as one would desire because there was no control over the temperature. The weekly average maximum temperature varied in the laboratory as much as 15 degrees between the hot summer months and the early fall.

Field plot tests were made on some of the more promising chemicals, where there was a large enough sample. The technique used was essentially that reported in previous experiments with the snail (McMullen, 1948; McMullen *et al.*, 1951). A total of 64,736 snails were used in these tests.

In both the laboratory and field tests comparisons were made with the results obtained under similar conditions with three known molluscacides: 2-cyclohexyl-4, 6-dinitrophenol; the dicyclohexylamine salt of this compound; and sodium pentachlorophenate (Berry, Hunter, Kuntz, McMullen, Stirewalt, *ibid.*; Pesigan, 1950).

In a project of this scope the assistance of many persons and organizations was required. First of all, to get financial support it was necessary to have a large number of samples available. The initial impetus was given by the interest and vision of Dr. Sterling Brackett, American Cyanamid Co. In each of the other organizations that submitted samples there was someone who gave invaluable assistance. Among these Dr. J. M. Magner, Monsanto Chemical Co., and Dr. F. W. Fletcher, Dow Chemical Co., should be mentioned. The project was financed by the Army Medical Research and Development Board, Contract No. DA-49-007-MD-39, O.I. No. 143–50. The active interest and advice given by Lt. Col. W. D. Tigertt, Army Medical Service Graduate School, was an important factor in this phase. Arrangements in Japan were made by various members of the Public Health and Welfare Section, GHQ, SCAP, and Dr. Y. Komiya, Department of Parasitology, National Institute of Health. The experimental work was done in the Department of Parasitology, Yamanashi Medical Research Institute, Kofu. The cooperation and assistance of Dr. S. Sugiura, head of the Department of Parasitology, and his staff were indispensable. In the field work the understanding and cooperation of the mura officials and the owners of the property where the experiments were carried out were excellent. The author is also greatly indebted to his assistants, Mr. T. Iijima, Miss K. Shinohara, Miss M. Sugiura, Mr. S. Komiyama, and Mr. John E. Simmons for their constant attention to monotonous details and the faithfulness with which they did their work.

RESULTS OF LABORATORY SCREENING

In a paper of this kind it is felt that the chemicals that show moderate and great toxicity are of primary interest. Chemists may be interested also in the less toxic compounds and even those that showed no molluscacidal activity. Presentation of data on these must await a more careful analysis.

Mixtures of 10 Chemicals—Out of the 413 mixtures submitted 33 were sufficiently active to indicate that tests of the individual chemicals were warranted. This initial screening eliminated 3,800 compounds and our attention was concentrated on the remaining 330. Unless some otherwise active chemicals had been inactivated in the mixtures this was a satisfactory method of screening. It was a rapid method of finding interesting compounds at a great saving of time and expense. When the separate compounds from the mixtures were tested, 12 contained two or more chemicals that were only slightly or moderately active. Ten of the mixtures were found to contain a single active compound of at least moderate toxicity. Eleven mixtures did not contain active compounds. The relatively large number in the last group may have been due to some synergistic action or to the fact that the amounts in the mixtures were not weighed and the quantity present may have been considerably more than in the accurately determined dilutions used in the separation tests. Out of the 330 chemicals tested in the sec-

ond series of experiments three were moderately toxic (Table 2), 14 were mildly toxic, 49 were slightly toxic and the remainder were non toxic.

Separate Samples—Tests on separate samples began with accurately determined amounts of the chemicals. Except for variations in the temperature the conditions were the same in all tests. Out of the 1675 samples, 576 showed measurable molluscacidal activity. Because of the temperature variations it is believed that the LD_{50} obtained for each of these active compounds cannot be taken literally. From experience it is possible to divide them into four categories. There were 18 compounds that were toxic, with an LD_{50} of 1:50,000 or above (Table 1). Thirty-six were moderately toxic (Table 2), with an LD_{50} of 1:25,000 to 1:45,000. Seventy-seven were mildly toxic with an LD_{50} of 1:6,000 to 1:20,000. The remainder of the active compounds should be considered as being only slightly toxic or questionably so.

Dual Mixtures—After determining some of the active compounds it was felt that some combinations might be more effective than the separate chemicals. Two types of mixtures were tried. In early tests it was noted that some chemicals were quite irritating to the snails. In some cases they were able to protect themselves from the material and the action was sometimes rather slow. Other chemicals, some toxic and others not, produced body extension. It was hoped that the combination of an irritating but highly toxic compound mixed with one that caused body extension would increase exposure. Various combinations of these two types of compounds were made. Five out of the 19 combinations gave an LD_{50} of more than 1:100,000 and were considerably more toxic than the separate chemicals (Table 1). The other mixture was made of solutions of copper sulphate and sodium pentachlorophenate. This produces copper pentachlorophenate in a form that is easy to distribute in a water suspension. Copper pentachlorophenate in any other form is difficult to handle because of its insolubility. This combination seemed to be a little more toxic than the copper pentachlorophenate alone but did not seem to be any better then sodium pentachlorophenate.

RESULTS OF FIELD PLOT TESTS

Most of the field testing had to be done during the summer months, not an ideal time, but some information was obtained on the relative effectiveness of some of the chemicals. For these tests there were large enough samples of 22 chemicals that had been sufficiently active in the laboratory to warrant trials in the field. The range in chemicals tested extended from those that were mildly toxic to those that were very active in the laboratory tests. A summary of the results will be found in Table 3. In most cases the results of the field tests correlated rather well with those obtained in the laboratory. In preparing the chemicals for application each one was dissolved in the best solvent available and then mixed in water for distribution. In some cases this did not make a mixture that was suitable for good distribution. With such chemicals a better method of distribution would undoubtedly increase their efficiency. Larger amounts of the ineffective chemicals probably would be toxic but it is doubted if they would be practical.

TABLE 1

A list of the compounds found to have the greatest molluscacidal activity in laboratory test

SURVEY NO.	CHEMICAL	SOURCE
	Dinitro-phenol compounds	
DOW-1	2-sec.-Butyl-4,6-dinitrophenol	(Dow)
K-1221	4,6-Dinitro-2-amyl-phenol	(Koppers)
Cr-1294	2,4-Dinitro-6-capryl-phenol	(Rohm & Haas)
K-1688	2,4-Dinitro-3-methyl-6-t-butylphenol	(Koppers)
K-1694	2,4-Dinitro-3-methyl-6-isopropylphenol	(Koppers)
DOW-3	2,4-Dinitro-6-phenylphenol	(Dow)
DOW-A	2-Cyclohexyl-4,6-dinitrophenol	(Dow)
DOW-B	"DN No. 1 (40% proprietary mixture of above)	(Dow)
DOW-C	2-Cyclohexyl-4,6-dinitrophenol, dicyclohexylamine salt	(Dow)
DOW-37	2-Isopropyl-4,6-dinitrophenol	(Dow)
DOW-30	"Premerge" (Alkanolamine salts of ethanol and isopropyl series of dinitro-o-sec-butylphenol, 54%)	(Dow)
	Halogenated phenol compounds	
IU-500	2-Chloro-o-phenylphenol	(Uni. Illinois)
K-1554	2,4,5-Trichlorophenol	(Koppers)
	Organic phosphorus compounds	
DOW-57	Restricted	(Dow)
CP-2323	Tetraisopropyl monothionopyrophosphate	(Monsanto)
	Miscellaneous compounds	
IU-262	Dibutyl-arsenic acid	(Uni. Illinois)
He-761	3,4-Dichlorophenyl-4-nitrobenzenesulfonamide	(Rohm & Haas)
IU-471	n-Nonyl piperidine hydrochloride	(Uni. Illinois)
	Dual mixtures	
DOW-A & CP-2408	2-Cyclohexyl-4,6-dinitrophenol & Dipropyl p-nitrophenyl thionophosphate	(Dow) (Monsanto)
DOW-A & CP-2323	2-Cyclohexyl-4,6-dinitrophenol & Tetraisopropyl monothionopyrophosphate	(Dow) (Monsanto)
DOW-A & CP-2466	2-Cyclohexyl-4,6-dinitrophenol & Ethylmethyl *p*-nitrophenyl thionophosphate	(Dow) (Monsanto)
DOW-C & CP-2323	2-Cyclohexyl-4,6-dinitrophenol, dicyclohexylamine salt & Tetraisopropyl monothionopyrophosphate	(Dow) (Monsanto)
DOW-C & CP-2466	2-Cyclohexyl-4,6-dinitrophenol, dicyclohexylamine salt & Ethylmethyl *p*-nitrophenyl thionophosphate	(Dow) (Monsanto)

TABLE 2

A list of compounds found to be moderately active as molluscacides in laboratory tests

SURVEY NO.	CHEMICAL	SOURCE
	Dinitro-phenol compounds	
K-1686	2,6-Dinitro-4-S-amylphenol	(Koppers)
K-1697	4,6-Dinitro-2-(*a*-methylbenzyl)phenol	(Koppers)
K-1763	2,4-Dinitro-3-methyl-5-ethylphenol	(Koppers)
K-1823	4,6-Dinitro-2-phenylphenol	(Koppers)
Lo-454	S-Cetyl-thiuronium-4,6-dinitro-2-caprylphenate	(Rohm & Haas)
DOW-D	"DN-111" (20% proprietary mixture of 2-cyclohexyl-4,6-dinitrophenol, dicyclohexylamine salt	(Dow)
	Halogenated phenol compounds	
DOW-40	Restricted	(Dow)
CP-2294	Restricted	(Monsanto)
NIH-674	Copper pentachlorophenate	(Nat. Inst. Heal., Dow)
DOW-41	Restricted	(Dow)
CP-1558	Restricted	(Monsanto)
Lo-432	S-Cetyl-N,N′-dimethyl-thiuronium-pentachlorophenate	(Rohm & Haas)
CP-2293	Restricted	(Monsanto)
CP-536	2,2′-Methylenebis(4,6-dichlorophenol)	(Monsanto)
CL-763	Pentachlorophenol	(Amer. Cyan. Monsanto, Dow)
X-6088-64	Restricted	(Hercules)
CP-A	Sodium pentachlorophenate	(Monsanto, Dow)
CP-2367	2,2′-Sulfonylbis(4-chlorophenol)	(Monsanto)
CP-5	2,3,4,6-Tetrachlorophenol	(Monsanto)
VAN-76	"Vanicide 76"—sodium salts of dimethyl dithio carbamic acid, 2-mercaptobenzothiazole, pentachlorophenol, and other chlorinated phenols.	(Vanderbilt)
	Nitro-phenol compounds	
DOW-48	4,4′-Isopropylidene bis(2-nitrophenol)	(Dow)
K-1828	4-Nitro-2,6-dimethylphenol	(Koppers)
K-1839	4-Nitro-2-phenylphenol	(Koppers)
DOW-38	2,4,5-Trichloro-6-nitrophenol	(Dow)
	Organic phosphorus compounds	
CP-850	Dibutyl phenyl phosphate	(Monsanto)
CP-849	Diethyl phenyl phosphate	(Monsanto)
CP-707	Ethyl di-(*p*-chlorophenyl) phosphinate	(Monsanto)
CP-1055-(5)	Tetraethyl dithionopyrophosphate	(Monsanto)
CP-847-R	Tetraethyl monothionopyrophosphate	(Monsanto)
	Miscellaneous compounds	
IU-219	Acetophenone pinacol	(Uni. Illinois)
IU-211	Arsenobenzene	(Uni. Illinois)
CL-3570	2-(*b*-Chloroethyl) benzimidazole hydrochloride	(Amer. Cyan.)
IU-331	Dibutylarsenic acid, copper salt	(Uni. Illinois)
CP-1388	4,5-Dichloro-3(2H)-pyridazone	(Monsanto)
CL-1448	N,N^2-dimethyl-N^2,N^4-diphenylmelamine	(Amer. Cyan.)
IU-396	Diphenyl mercury	(Uni. Illinois)
CP-2292	Restricted	(Monsanto)
CP-203	2-Pentachlorophenoxyethanol	(Monsanto)
IU-204	*p*-Tolyl mercuric chloride	(Uni. Illinois)

TABLE 3

A summary of the results obtained with molluscacidal compounds in field plot tests

CHEMICAL OR SURVEY NO.	ACTIVITY IN PLATE TEST	FIELD TESTS: 25 FT SECTION OF DITCH	
		Effective dosage or maximum amount used	Results
N,N-Amylcyclohexylbenzylamine (Monsanto)	Mild	40 cc	Ineffective
X-Chloro-o-nitrobiphenyl (Monsanto)	Mild	58 cc	Effective
Ethyl di-(*p*-chlorophenol) phosphinate (Monsanto)	Moderate	40 gms	Ineffective
Tetraethyl monothionopyrophosphate (Monsanto)	Moderate	40 gms	Ineffective
Di-(2-chloroethyl)*p*-nitrophenyl thionophosphate (Monsanto)	Mild	60 cc	Moderately effective
Tetrapropyl monothionopyrophosphate (Monsanto)	Mild	46 cc	Moderately effective
Tetraethyl dithionopyrophosphate (Monsanto)	Moderate	40 cc	Effective
Tetraisopropyl monothionopyrophosphate (Monsanto)	Toxic	40 cc	Moderately effective
1-(3,4-Dichlorophenyl) ethanol (Monsanto)	Mild	75 cc	Moderately effective
2-Butoxyethyl-o-chlorophenyl phenylphosphate (Monsanto)	Mild	75 cc	Moderately effective
"Premerge" (Dow)	Toxic	10–20 gms	Effective
2-Isopropyl-4,6-dinitrophenol (Dow)	Toxic	5–10 gms	Effective
2,4,5-Trichloro-6-nitrophenol (Dow)	Moderate	20 gms	Effective
DOW-40 (Dow)	Moderate	50 gms	Effective
DOW-41 (Dow)	Moderate	20–50 gms	Effective
4,4′-Isopropylidene bis(2-nitrophenol) (Dow)	Moderate	20 gms	Ineffective
DOW-57 (Dow)	Toxic	10–20 gms	Effective
Pentachlorophenol (Amer. Cyan., Monsanto, Dow)	Moderate	35 gms	Effective
"Vanicide 76" (Vanderbilt)	Moderate	40 gms	Effective
2,4-Dinitro-6-capryl phenol (Rohm & Haas)	Toxic	20 cc	Effective
3,4-Dichlorophenyl-4-nitrobenzenesulfonamide (Rohm & Haas)	Toxic	20 gms	Effective
S-Cetyl-thiuronium-4,6-dinitro-2-capryl-phenate (Rohm & Haas)	Moderate	50 gms	Effective
2-Cyclohexyl-4,6-dinitrophenol (Dow) & Tetra-isopropyl monothionopyrophosphate	Toxic	5 gms 1–5 cc	Effective
2-Isopropyl-4,6-dinitrophenol & Dow-57	Toxic	10 gms 10 cc	Effective
Sodium pentachlorophenate (Dow, Monsanto) & copper sulfate	Moderate	20–50 gms 8–20 gms	Effective

DISCUSSION

Tables 1 and 2 show that certain groups of chemicals are well represented. Phenol compounds predominate and these can be divided into three types. The dinitrophenols are the most active and many of them are found in Table 1. Only two of the halogenated phenol compounds appear in this list. A few of the nitrophenols appear in the list of moderately active compounds (Table 2).

Many compounds related to these three types are to be found among the less or non toxic samples tested. Two organic phosphates appear in the list of the most toxic compounds (Table 1) and five are to be found in the moderately toxic group (Table 2). Only one arsenic compound was active enough to be included among the more active compounds and two were moderately toxic. Two mercury and two copper compounds were moderately toxic. Two compounds 3,4-dichlorophenyl 4-nitrobenzenesulfonamide and n-nonyl piperidine hydrochloride, were quite toxic but many related compounds were not. Among the moderately toxic compounds appear acetophenone pinacol, 2-pentachlorophenoxyethanol, 4,5-dichloro-3-pyridozone, 3-ethylamino propionitrile, N,N²-dimethyl-N²,N⁴-diphenylmelamaine, and 2-(*b*-chloroethyl) benzimidazole hydrochloride. In some cases these gave very irregular results and many related compounds were less or non toxic.

In the field experiments reported here the inverse ratio of amount of chemical and the per cent kill is best among the dinitrophenols, but other compounds may prove to be more practical. The primary problem for the chemists and commercial organizations is to suggest suitable formulations of the active compounds that will be economically feasible and relatively safe to handle. The best combination probably would be one that could be mixed with water for spraying. It may be that some of the less active compounds can compete with those that are very active. This would depend on the cost of effective amounts. For example, in previous experiments (McMullen *et al.*, 1951) it was found that sodium pentachlorophenate can compete with 2-cyclohexyl-4,6-dinitrophenol even though approximately four times as much is needed to get comparable control.

The author believes that while molluscacides would be the best method for reducing schistosomiasis to a minimum under combat conditions, they are not the final answer in the long term control of schistosomiasis in civilian populations. Their use is simply a facet, but a very important one, of a very intricate problem. It is known that even after a series of applications of an effective molluscacide the few snails that remain can soon repopulate an area (McMullen *et al.*, 1951; Hunter *et al.*, 1951). For this reason a control program under normal conditions should be a long enough one and take into account all the known methods of reducing the opportunity of infection. This involves problems in engineering, agriculture, disposal or treatment of definitive hosts, and sanitation as well as the use of molluscacides. Anything less than a total effort is doomed to failure.

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SCHISTOSOMIASIS IN AN ENDEMIC AREA IN THE DOMINICAN REPUBLIC[1]

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Ponce Pinedo (1945) was the first to report the existence of autochthonous cases of schistosomiasis mansoni in the Dominican Republic, and later (1947) he was able to demonstrate that an endemic focus of the disease exists in the vicinity of Hato Mayor, Seibo Province. He succeeded in collecting infected *Australorbis glabratus* from two streams near the town of Hato Mayor and recovered adults and eggs of *Schistosoma mansoni*, after exposing rabbits to the cercariae from these snails. However, the extent and seriousness of the endemic focus at Hato Mayor has not been known, and there has even been doubt in some quarters that the parasite is actually established there.

In March of 1951 one of us (CMV) collected *Australorbis glabratus* from the arroyo Paña Paña near Hato Mayor and recovered from some of them cercariae tentatively identified as *Schistosoma mansoni*. This collection aroused interest in the endemic focus of the disease at Hato Mayor, and on the initiative of Dr. Thomas T. Mackie, Director, Institute of Tropical Medicine, a cooperative project was set up to investigate the problem. Consequently, in the summer of 1951 new information was obtained concerning the present status of the disease in that area. A study was made of the distribution of *A. glabratus* in the vicinity of the town and the prevalence of *S. mansoni* infection in these snails. The prevalence of *S. mansoni* infection in children of the town was also determined. The results of this study are reported here.

Hato Mayor is a town of about 4,000 people situated 38 kilometers by road to the north of San Pedro de Macorís in the eastern part of the Dominican Republic. It lies in the foothills of a low mountain range and is surrounded principally by grazing land. There is no irrigation in the vicinity, and the water supply for the town comes by pipeline from the hills to the north.

At the southeast corner of the town a small stream, the arroyo Las Guamas, flows into a somewhat larger stream, the arroyo Paña Paña (Figure 1). The Las Guamas arises in flat land immediately north of the town. Most of the time there is no flowing water in the upper portions of the Las Guamas, but the stream bed contains numerous small, permanent pools containing water lilies and other aquatic vegetation. From a point north of the road to El Cercado de San Fran-

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cisco to its junction with the Paña Paña, the Las Guamas has been incorporated into a system of open concrete drains installed for purposes of malaria control (Figure 2). Ordinarily this portion of the stream carries very little water, and of course there is no vegetation in it. In times of heavy rains the Las Guamas carries a large volume of flood water into the Paña Paña.

The arroyo Paña Paña arises north of Hato Mayor as a series of dry washes which, during rains, receive water from the hills to the north. Farther down the drainage system the rocky stream bed has scattered permanent pools of water

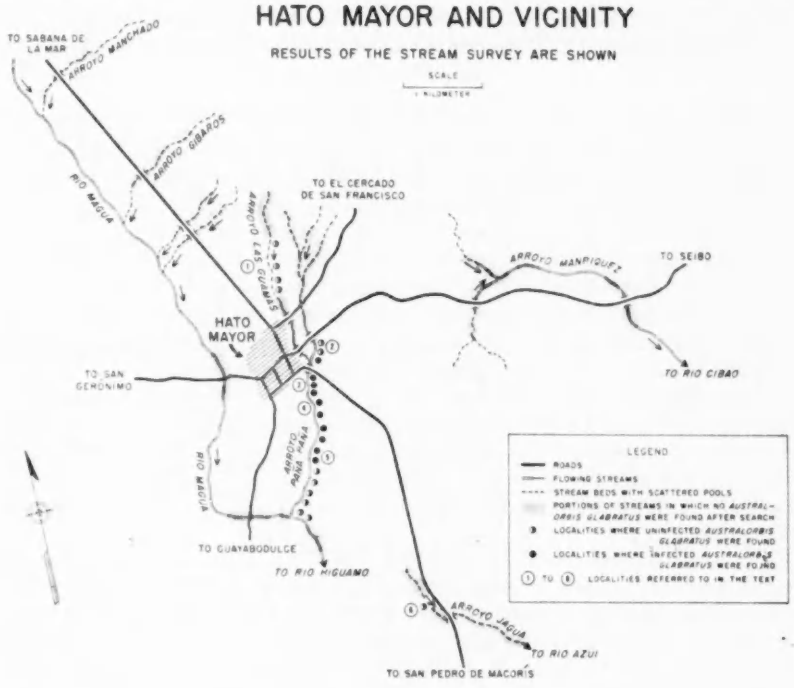

FIG. 1

except when the stream is in flood. Downstream from the place where it crosses the road to El Cercado de San Francisco, the stream bed contains water throughout the year, and there is a small flow of water even in the dry periods. The stream bed, throughout its length, is subject to flooding and harbors only a small amount of vegetation. The portion of the Paña Paña flowing close to Hato Mayor shows evidence of considerable fecal pollution. Some of this pollution probably comes from the Las Guamas during heavy rains since the portions of the drains of the latter passing under bridges are used as defecation areas.

At the point where the Paña Paña is nearest to the town (area 3 in Figure 1),

it flows for a short distance through an open, grassy, treeless area where the banks of the stream are fairly low. This area, just downstream from the bridge on the road to San Pedro de Macorís, is unfenced and readily accessible from the town. Children of the town play along the stream and sometimes wade in it. Mounted travelers and pedestrians cross a shallow, rocky portion of the stream bed in this area. Apparently the Paña Paña near the town is little used for bathing and for washing clothes.

There is a fence across the stream about 40 meters below the bridge, and at this point the stream leaves the open grassy area and flows under heavy shade between steep, high banks. From this point all the way to its junction with the Rio Magua, the stream flows through private lands and relatively few people have access to it.

DISTRIBUTION OF *Australorbis glabratus* IN THE VICINITY OF HATO MAYOR

At the outset of the study an attempt was made to determine the limits of the distribution of *A. glabratus* in the vicinity of Hato Mayor. First, the streams in close proximity to Hato Mayor were searched for the snails. The portions of the streams searched and the results are shown in Figure 1.

All of the arroyo Paña Paña except parts of the dry washes in its headwaters was searched thoroughly for snails. *A. glabratus* was found all the way from its mouth to the point where the Seibo Road crosses it east of Hato Mayor. The snails were most numerous in the areas designated by numbers 3 and 4 in Figure 1. Relatively few snails were found in areas 2 and 5 in Figure 1.

The arroyo Las Guamas was searched by tracing its course upstream until no water could be found in the stream bed. The portion of the stream near Hato Mayor could harbor no snails since it is paved with concrete and carries very little water except following heavy rains. However, above the drain in flat pasture land partly shaded by trees, the small, permanent pools had small numbers of fully mature *A. glabratus*.

Parts of all the other streams near Hato Mayor were also searched for snails and as shown in Figure 1, *A. glabratus* was not found in any of them except that one snail was found in the arroyo Jagua, which arises from springs south of Hato Mayor. Portions of numerous other streams at distances up to 20 kilometers to the west and south of Hato Mayor were also searched for *A. glabratus*. These streams included the Rio Azui into which the arroyo Jagua flows, the Rio Magua, the Rio Higuamo into which the Magua flows, the Rio Casui, and many of their tributaries. No *A. glabratus* were found in any of these streams except for the few snails found in the Magua near the mouth of the Paña Paña.

COLLECTIONS OF SNAILS HARBORING *Schistosoma mansoni*

A. glabratus collected in the arroyo Paña Paña and the arroyo Las Guamas were brought into the laboratory, isolated in small bottles, and those that shed cercariae were set aside and the cercariae identified. Most of the snails that failed to shed cercariae were crushed and the snail bodies examined for trematode infection. Additional infections were found in this way (Table 1).

Many of the snails collected from that portion of the Paña Paña flowing near the town of Hato Mayor (area 3, Figure 1) were infected with cercariae tentatively identified on morphological grounds as *Schistosoma mansoni*. Snails collected from the Paña Paña above and below area 3 were also infected, but the proportion of infected snails was smaller. On the other hand, none of the snails from the pools of the Las Guamas was infected.

EXPOSURE OF MICE AND THE RECOVERY OF SCHISTOSOMES

In order to verify the identification of the schistosome as *S. mansoni*, white Swiss mice were exposed to the cercariae and examined 55 days later. Numerous typical eggs and adults of *Schistosoma mansoni* were recovered from the mice.[2]

TABLE 1

Record of snail collections made in the vicinity of Hato Mayor in June and July of 1951

DESCRIPTION OF THE COLLECTION AREA	LOCALITY SEE FIG. 1	DATE OF COLLECTION	TOTAL SNAILS COLLECTED	SNAILS FOUND INFECTED BY ISOLATION	SNAILS FOUND INFECTED BY DISSECTION	TOTAL SNAILS INFECTED	PER CENT INFECTED
The Las Guamas pools	1	7/11/51	63	0	0	0	0
Paña Paña between Seibo Road and San Pedro Road	2	7/11/51	60	1	0	1	2
Paña Paña below San Pedro Road.	3	6/27/51	307	124	—	124	40
Paña Paña below San Pedro Road.	3	7/10/51	94	13	—	13	14
Paña Paña below San Pedro Road.	3	7/14/51	142	30	3	33	23
Paña Paña below San Pedro Road.	3	7/24/51	196	10	22	32	16
Paña Paña below area 3 and separated from it by a fence	4	7/24/51	63	3	8	11	17
Paña Paña midway between San Pedro Rd. and the Magua Rd.	5	7/10/51	118	2	0	2	2
The Arroyo Jagua	6	7/19/51	1	—	0	0	0

EXAMINATION OF STOOLS FROM CHILDREN OF HATO MAYOR

The portion of the arroyo Paña Paña which harbored the greatest concentration of *S. mansoni*-infected snails flows close to the town of Hato Mayor and almost all of the people living near the stream at that point live within the limits of the town. To arrive at an estimate of the prevalence of *S. mansoni* infections in the people having access to this portion of the stream, stools from children living in the town were examined for eggs of *S. mansoni*. Through the interest and cooperation of the Ministry of Health, the Sanitary Inspector in Hato Mayor collected and delivered to us stools from 243 children between the ages of 5 and 15 inclusive, probably representing a fair sample of the juvenile population of the town. A portion of each stool was examined for worm eggs after concentration by an acid-ether technique in which Triton NE was used substantially as described by Weller and Dammin (1945). The sediment from each tube

[2] We wish here to express appreciation to Captain W. G. Jahnes, U. S. Army Medical Department, Tropical Research Medical Laboratory, San Juan, Puerto Rico for aid in procuring mice.

TABLE 2

S. mansoni infections found in 243 children of Hato Mayor by age and sex; 112 of the children were males, 131 were females

AGE (YRS.)	NO. OF CHILDREN EACH AGE	POSITIVE STOOLS EACH AGE	BOYS POSITIVE EACH AGE	GIRLS POSITIVE EACH AGE
5	47	6	3	3
6	26	0	0	0
7	29	7	3	4
8	25	4	2	2
9	24	6	6	0
10	35	5	1	4
11	10	4	2	2
12	29	7	4	3
13	7	5	2	3
14	3	2	1	1
15	2	2	0	2
Unknown	6	4	2	2
Total.....	243	52	26	26

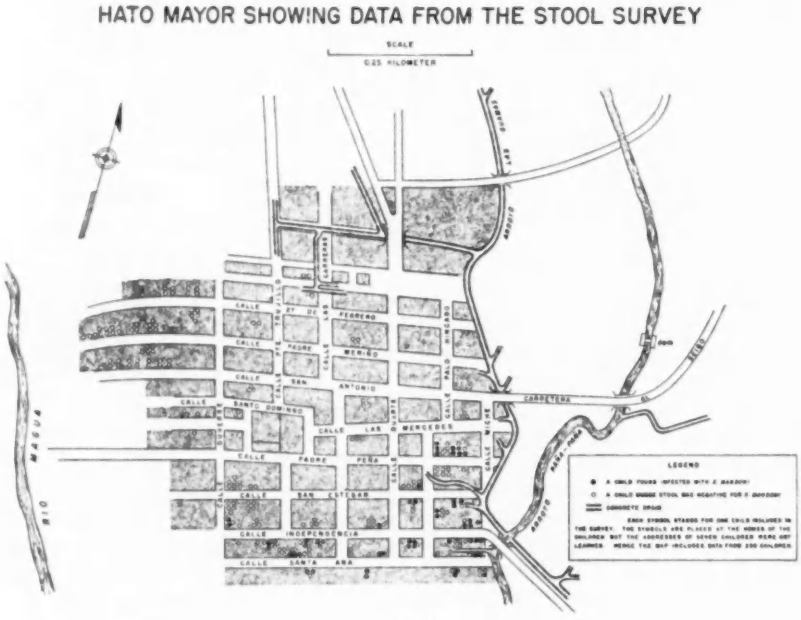

FIG. 2

was pipetted onto two glass slides and covered with 22 by 40 mm. coverglasses. All the material under both covers was searched before a stool was declared negative.

Data from the stool examinations are presented in Table 2. Characteristic eggs of *S. mansoni* were found in 52, or 21.4 per cent, of the stools examined. Half of the infected stools were from boys and half from girls; the prevalence in the 112 boys and 131 girls included in the survey, therefore, was approximately equal in both sexes. Infected children were found at all ages from 5 to 15 except in the six-year-old group.

Figure 2 is a map of Hato Mayor on which symbols have been placed to mark the residences of 236[3] children included in the stool survey. The symbol locating a child also indicates whether he was found infected or not by the stool examinations. It may be observed that almost every child whose stool was positive lived in the southeast corner of the town near the arroyo Paña Paña.

DISCUSSION

Australorbis glabratus was found in the arroyo Las Guamas, the arroyo Paña Paña, the Rio Magua, and the arroyo Jagua. Only one snail was found in the Jagua, and so it is not possible to say, at present, that the species is established in that stream. There can be no doubt that *A. glabratus* is breeding in the arroyo Paña Paña between the Seibo Road and the area marked number 5 on Figure 1. Adult snails were numerous, and both juvenile snails and snail eggs were found in that part of the stream both in March and in the summer months. Since few of the snails were found in the lower Paña Paña and in the Magua, it is possible that the snails are not breeding in those places but occasionally are washed into that area when the stream is in flood.

It is clear that *A. glabratus* is also breeding successfully in the pools of the Las Guamas. Before the lower Las Guamas was made into a part of the concrete drainage system the whole length of the stream from its mouth at the Paña Paña to the pools marked on Figure 1 probably harbored *A. glabratus*. At present the snails in the upper Las Guamas appear to be a residuum of a larger population once spread along the stream. However, the snails now in the Las Guamas are not in an area much frequented by people, and are probably not a direct threat to the health of the people of Hato Mayor. Indirectly they may contribute to the problem since some of the snails may be washed downstream and reinforce the snail population of the Paña Paña.

It is concluded that *A. glabratus* probably has a very restricted distribution in the part of the Dominican Republic that was studied. Indeed, it is quite possible that the distribution of *A. glabratus*, in the region studied, is limited to the Las Guamas-Paña Paña drainage and to a segment of the Magua near the mouth of the Paña Paña. Much more extensive stream survey work is needed to determine this with any degree of certainty.

The apparently limited distribution of *A. glabratus* in the eastern part of the Dominican Republic implies a limited distribution of human infections with *S. mansoni*, and this seems to be the case, since the only known autochthonous cases of schistosomiasis mansoni in the Dominican Republic have come from Hato Mayor (Ponce Pinedo, 1945, 1947; and Imbert, 1946).

[3] The addresses of 7 of the 243 children included in the survey were not provided.

The greatest concentration of infected snails in the Paña Paña is near the town of Hato Mayor. This is the only part of the stream to which the people have ready access, and it is probably the area in which practically all of the *S. mansoni* infections in the people of the town are acquired. Small numbers of farming people living or working along the lower Paña Paña below the town are also liable to exposure.

The results of the stool survey give ample evidence that a serious endemic focus of *S. mansoni* infection exists in the town of Hato Mayor. The population of the town, according to the census of 1950, was 3,911, and it is estimated that about 28 per cent, or about 1,100 of these persons were children between the ages of 5 and 15 inclusive. If one takes the figure of 20 per cent as indicating the true prevalence of infection in the children of the town, then about 220 children in that age group in Hato Mayor may be infected. Actually, since some infections are surely missed when only a single stool is examined by the acid-ether technique (Hernández Morales *et al.* 1950), it is reasonable to assume that well over 20 per cent of the children of the town between 5 and 15 are infected. It is noteworthy that almost every child whose stool was positive in the survey lived in the part of the town close to the Paña Paña. Apparently proximity of a child's home to the stream greatly increased the chances that he would become infected.

Ponce Pinedo (1947) found only a very small number of infections in an extensive series of stools examined by a sedimentation technique. Detection of a much larger number of infections in the present study could be interpreted to mean that the number of infections in Hato Mayor has increased in recent years. However, since the techniques for examination of the feces differed greatly in the two studies and the populations selected for study were also different, it is not possible to attach significance to the difference in results.

It has long been felt that the best way to attack schistosomiasis in an endemic area is to destroy the snail intermediate hosts and thereby interfere with the transmission of the parasite from man to man. The restricted endemic area of schistosomiasis mansoni in the vicinity of Hato Mayor seems to provide an excellent testing ground for the control of endemic schistosomiasis by the use of molluscacides.

SUMMARY

A study was made of the endemic area of schistosomiasis mansoni at Hato Mayor in the Dominican Republic. *Australorbis glabratus*, the snail host for *Schistosoma mansoni*, seems to have a very restricted distribution in the vicinity of Hato Mayor. Numerous *A. glabratus* were found in streams close to the town but none was found in other streams up to 20 kilometers from the town. Many of the snails harbored cercariae which developed into adult *S. mansoni* in mice. Examination of a single stool from each of 243 children between the ages of 5 and 15 living in Hato Mayor revealed 52 infections with *S. mansoni*.

It is concluded that a serious endemic focus of schistosomiasis mansoni exists in the vicinity of Hato Mayor, and that the disease is being acquired by a relatively large proportion of the children of the town. Because the endemic area is

apparently restricted to narrow geographic limits, it is considered especially well suited for a study on the use of molluscacides in the control of schistosomiasis mansoni.

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TREATMENT OF ASCARIASIS IN CHILDREN WITH HETRAZAN® SYRUP[1]

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The first evidence of the ascaricidal property of 1-diethylcarbamyl-4-methylpiperazine hydrochloride (Hetrazan®) was reported by Hewitt and his associates (1947). These investigators gave doses of 25 mg. per Kg. of body weight to dogs with ascariasis and experienced 100 per cent cures. The numerous studies subsequently reported on *Ascaris lumbricoides* in humans, beginning with that of Oliver-Gonzáles and associates in Puerto Rico (1949), reveal variability of results in different geographical locations. Optimistic statistics have been prepsented from Puerto Rico, the Phillippines (Garcia 1949), Mexico (Ruiz 1949; Ruiz *et al.* 1950), Tennessee (Ettledorf *et al.* 1950) and the Caribbean (Loughlin *et al.* 1951). Less satisfactory results were encountered in other studies in Mexico (Hewitt *et al.*), in the Belgian Congo (Wanson 1949), in the Gold Coast (Colbourne 1950) and in Honduras (Hoekenga 1951).

Some of these investigations with less promising results employed dosages equivalent to or even higher than the studies with favorable outcome. Several explanations for this apparent inconsistency are suggested. Perhaps there is a geographical variation in the susceptibility to diethylcarbamazine (Hetrazan) of *Ascaris lumbricoides* strains. Differences in criteria of cure may account for some of the deviations. Or, the discrepancy may be due in part to differences in dosage used, although, as just pointed out, this would not seem to be the principal factor. Fourthly, unrecognized seasonal variations in worm burden may have caused an apparently higher rate of cure in some studies than others, in as much as worm burdens are at the lowest level at the end of the dry season and highest just after the rainy season.

In areas where the incidence of ascariasis is high, it is not practical to continue treatment for an extended time. An ascaricidal compound requiring prolonged administration would have to have significant advantages over other vermifuges to commend its use at all. Diethylcarbamazine does have certain advantages in its favor, such as its lack of toxicity in therapeutic doses and its effectiveness against ascaris without fasting or purgation. It would be highly desirable to be able to combine these advantages with therapeutic efficacy when the drug is given in a large single dose or in fairly heavy amounts over a short period of time.

In a previous clinical trial (Hoekenga 1951) we attempted to ascertain whether either single-dose or short-term treatment of ascariasis would be effective and practical in Honduran adults. We discovered that 900 mg. of diethylcarbamazine, when given in three divided doses on one day, resulted in only 37.5 per cent of

[1] The Hetrazan® Syrup was supplied by the Lederle Laboratories Division of the American Cyanamid Company.

cure in 8 patient; 1.0 Gm. when given in a single dose to 15 persons resulted in only 33.3 per cent cure; 1.8 Gm. when given in divided doses over a three day period to 15 patients gave a 60 per cent cure rate. It was concluded that in such quantities, it was not a suitable drug for mass treatment of ascariasis, but that when given for longer periods it could be valuable for treatment of ascariasis in debilitated persons and young children.

In the present experiment, we have utilized a highly palatable preparation of Hetrazan Syrup for the treatment of ascariasis in children. We were stimulated to do this as a result of a communication (Loughlin *et al.* 1951) indicating that elsewhere in the Caribbean area administration of such a syrup reduced the egg count by more than 90 per cent. The dose used was 6 mg. of diethylcarbamazine per pound of body weight once a day for 4 days.

MATERIAL AND METHODS

Study No. 1. This experiment comprised 95 mestizo children 2 to 7 years of age, all resident in banana farm communities located 2 to 4 miles from La Lima, near the north coast of Honduras. Forty-five were females and fifty were males. All of the children were treated on the same dosage schedule initially. Syrup containing 30 mg. of diethylcarbamazine per cc. was employed. Each child was given 6 mg. per pound of body weight per day for four days. The drug was administered in the early morning but the fasting state was not required. The measured amount was diluted in 50 to 100 cc. of water and taken in a single dose. Purgation was not employed either before or after treatment. Post-treatment fecal examinations were made one week after completion of treatment and again two to three weeks after treatment. Egg counts were not done. The criterion for cure was the complete absence of ova from concentrated (zinc sulfate flotation) stools on both of the post-treatment studies. A large group of the children who failed to achieve negative stools on this initial regimen was re-treated with the identical dosage and method; this re-treatment was commenced three weeks after the completion of the first treatment course. A group of the children who again failed on the second treatment was given the Hetrazan Syrup in identical manner a third time; this was administered three weeks after completion of the second course of treatment.

Study No. 2. Thirty children of similar race and age were given Hetrazan Syrup in double the amount employed in Study No. 1, i.e, they received 12 mg. per pound of body weight per day for four days. The manner of administration and the criteria of cure were the same as that described above. None of the children in this study were re-treated.

RESULTS

Study No. 1. The initial four-day regimen resulted in cure in only 36 per cent of the 95 children. Thirty per cent of those given a second course of the drug developed negative stools. Three-fourths of those given a third course were cured (Table 1). Although only 36 per cent of the children achieved negative stools on the first treatment course, the number who were known to have

passed adult ascaris was considerably higher—74 per cent. Similar passage of adult worms occurred after the second and third treatment courses. Thus, there was an obvious effect against the worms in most treatment courses, even though complete clearance from the intestinal tract occurred in a more limited number. As mentioned before, precise ova counts were not done, but it was a frequent impression that even in those who failed by the criteria used, there was a marked reduction in the number of ova seen on microscopic examination.

Study No. 2. This regimen resulted in a cure of 80 per cent (24 of the 30 children). Four of the six children who did not achieve negative stools did pass some adult ascaris worms nevertheless (Table 1).

Toxicity. The adult ascaris are passed alive after diethylcarbamazine treatment. This fact is possibly in some measure responsible for the infrequent toxic effects noted in the course of the various reported studies. Because they are not

TABLE 1

Treatment of ascariasis in children with Hetrazan® Syrup.[1]

DOSE	NO. TREATED	NO. WHO PASSED WORMS	NO. WITH NEGATIVE STOOLS	PER CENT WITH NEGATIVE STOOLS
Study No. 1				
First course: 6 mg/pound/day for 4 days	95	70	34	36
Second course: original dose repeated on failures from first course	40	32	12	30
Third course: original dose repeated on failures from second course	20	15	15	75
Study No. 2				
12 mg/pound/day for 4 days	30	25	24	80

[1] Results are based on examinations done one week and two weeks after completion of treatment.

yet dead, there is not the danger of absorption of toxic by-products from the disintegrating worms. In the present study, no toxic effects were noted with the therapeutic quantities employed.

COMMENTS

Many drugs will temporarily interrupt the egg-laying of the ascaris worms, and if examination is done too quickly after treatment, a "cure" may be apparent when it is not real. In some of the reported studies, stools were examined during the first three days; this is not reliable and is a possible clue to some of the discrepancies noted in these reports. Even examinations one week after treatment may give misleading negative results; about 10 per cent of our cases were negative on the one-week examination, but positive again on the two, week examination. This does not mean that additional worms were present-

but that egg-laying had been resumed by female worms not expelled by the treatment.

Probably there would have been fewer cures recorded in this study if we had been able to perform egg counts or at least done direct smears in addition to the zinc sulfate procedure. Performance of egg counts possibly would have been conducive to more exhaustive searching of slide preparations in some instances, and examination of direct smears may sometimes reveal unfertilized eggs which are passed in fairly high percentage when worm burdens become low and which oftentimes do not float on zinc sulfate.

It is important to indicate the season of the year during which these studies were undertaken. Study No. 1 was commenced at the end of the rainy season, when the worm burden is known to be at a high level; the second re-treatment occurred well towards the middle of the dry season, at a time when the worm burden would be spontaneously declining, and therefore the three-fourths cure rate noted must be interpreted with this fact in mind. Study No. 2 was begun in the middle of the dry season (again, when the worm burden would be on the decline), and consequently the 80 per cent rate noted with the double dose must be interpreted in this light. Just how much this seasonal variation in ascaris egg output has influenced our apparent cure rates is not known.

Animal experiments (Hewitt *et al.* 1948; Harned *et al.* 1948) demonstrate that diethylcarbamazine is excreted extremely rapidly, is not irritating to intestinal mucosa, and produces no tissue pathology in therapeutic doses. Theoretically, then, large single doses might be practical. Clinically, however, toxic effects sometimes occur; Oliver-González and his associates (1949) report that "single doses of 8 to 10 mg./Kg. produce quite severe malaise, vasoconstriction and nausea". We encountered no severe toxic reactions with the administration of 21 mg. per pound (26.4 mg./Kg.) of body weight per day, although several children were irritable and a few became nauseated. Five children who were given an experimental dose of 18 mg. per pound (39.6 mg./Kg.) of body weight all developed malaise; two of them vomited and one became drowsy and lethargic for several hours after the first administration. This larger amount is therefore not recommended.

SUMMARY

One hundred and twenty five Honduran children were treated for ascariasis with 1-diethylcarbamyl-4-methylpiperazine hydrochloride (Hetrazan®) in a syrup which contained 30 mg. of the drug per cc. Of the dosages employed, the most effective was 12 mg. per pound of body weight daily for 4 days; in 80 per cent of 30 cases this amount resulted in absence of ascaris eggs from concentrated (zinc sulfate flotation) stools on both of two post-treatment examinations. Smaller amounts gave less satisfactory results. Eighteen mg. per pound of body weight appeared to be too toxic.

Factors effecting the apparent cure rate are discussed. The most important are the criteria of cure and the season of the year during which the study is done.

It is concluded that when it is undesirable to fast or purge, diethylcarbamazine

may be a satisfactory vermifuge. It should be of particular advantage in private practice. However, it is not an ideal compound for mass treatment because multiple doses are necessary.

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THE DISTRIBUTION OF CERTAIN LIQUIDS IN THE ESOPHAGEAL DIVERTICULA AND STOMACH OF MOSQUITOES

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The esophageal diverticula in mosquitoes are three sacs attached to the esophagus, two small ones lying in the thorax dorsad to the esophagus, and a large one, the crop or ventral diverticulum, in the forepart of the abdomen, ventrad to the midgut, or stomach.

Much has been written and many conclusions drawn about the function of these structures and about the distribution of liquids ingested. All of the available literature has been reviewed, but for the most part, only those articles will be discussed which present adequate experimental evidence on the distribution of liquids in the midgut and diverticula.

The morphology, histology, and physiology of mosquito diverticula have been the subject of much investigation and speculation since Dufour's paper appeared in 1851. Nuttall and Shipley (1903), who laid the groundwork for subsequent morphological studies, considered the organs "food reservoirs", and succeeding investigators have varied in their opinions as to whether the function was that of storage, digestion, or both. MacGregor (1930, 1931) suggested the additional function of "air separators".

Investigations into the distribution of blood and other liquids in the alimentary tract of the mosquito have followed two main lines, one involving the manner in which the insect feeds, the other, the type of food itself.

Mosquitoes feed in two ways, (1) the females pierce and suck (bite) to obtain blood, and (2) males and females imbibe liquids from open drops or saturated pledgets, a method described as "discontinuous suction" by MacGregor. A female mosquito, biting through the skin of a host or through a membrane, may be seen to push back the labium and so unsheath the proboscis. A mosquito feeding by "discontinuous suction" dips and withdraws only the tip of the proboscis; the proboscis remains intact.

Methods of feeding devised for use in the laboratory have included those described as "forcible" (Fülleborn 1908, Martini 1925, Kadletz and Kusmina 1929, and Roy and Ghosh 1940); "artificial" (the Hertigs 1927); and the "unsheathed capillary tube technique" of MacGregor (1930).

Data on experimental work, or theories, dealing with the distribution of blood meals have been offered by de Grandpré and Charmoy (1900), Nuttall and Shipley (1903), Lebredo (1904), Schaudinn (1904), Eysell (1924), Patton and Cragg (1913), Genna (1920, 1921), Martini (1923), Wright (1924), Grassi (1924), Falleroni (1926), James (1926), Roy (1927), de Boissezon (1930), Philip (1930), MacGregor (1927, 1930, 1931), MacGregor and Lee (1929),

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Marshall and Staley (1932), Pawan (1937), Roy and Ghosh (1940), Bishop and Gilchrist (1946), and Lumsden (1947). Only three of these authors substantiated their claims with supporting data sufficient to be convincing.

Falleroni (1926) fed and dissected 284 *Anopheles maculipennis* before drawing the following conclusions:

1. *An. maculipennis* killed in different stages of biting, never showed more than traces of blood in the diverticula.

2. Blood taken by bite after the mosquito had fed on sugary solutions was always dispatched to the stomach.

3. *An. maculipennis* biting during the digestion of blood, dispatched the blood to the stomach and not to the diverticula, thus excluding the premise that the diverticula were used as reservoirs for blood.

4. If mosquitoes were allowed to bite when the abdomen was full of mature eggs, the blood was immediately dispatched to the stomach.

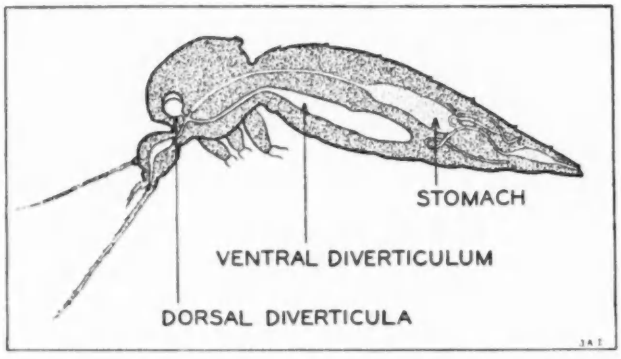

FIG. 1. Longitudinal section through a mosquito, showing a portion of the digestive tract.

Bishop and Gilchrist (1946) after allowing 18 *Aedes aegypti* to imbibe whole blood from open drops and 74 to feed through a membrane, dissected them immediately, and concluded that the primary destination of blood, whether taken from drops or through a membrane, was the stomach.

Lumsden (1947) dissected and examined 390 *Ae. aegypti* which had been allowed to bite until satiated, and concluded that in mosquitoes of this species, the blood was dispatched primarily to the stomach. A high percentage of those completing only a partial blood meal had blood in the diverticula; this was attributed to probable regurgitation from the stomach.

The fate of sugary solutions and liquids other than blood has been discussed by Nuttall and Shipley (1903), Eysell (1924), Genna (1920, 1921), Martini (1923), Wright (1924), Grassi (1924), Missiroli (1925), Falleroni (1926), MacGregor (1927, 1930, 1931), de Boissezon (1930), Yoeli and Mer (1938), Roy and Ghosh (1940), Bishop and Gilchrist (1946); only four of these authors have presented conclusive data.

Falleroni (1926) dissected 258 *An. maculipennis*, which had fed on sugary solutions, before he concluded that "free liquids are always pushed into the esophageal diverticula" to remain there about two days.

Marshall and Staley (1932) dissected 45 *Culex pipiens*, and later, some *Aedes detritus* which had fed on raisin juice. These results, together with some experiments on unspecified mosquitoes, led the authors to state that a meal of fruit juice was dispatched to the esophageal sacs.

Roy and Ghosh (1940) fed liquids other than blood to *Anopheles subpictus*, *An. stephensi*, *Ae. aegypti*, and *Culex fatigans* (= *quinquefasciatus*). They used the capillary tube technique in feeding 441 mosquitoes and found that the distribution of the liquids was independent of the species of mosquito, the kind of food, or the mode of feeding. When mosquitoes were fed liquids from cotton wool, the results were consistent, and the authors concluded that water and sugary solutions so imbibed were always dispatched to the diverticula.

Bishop and Gilchrist (1946) studied the destination of sugary solutions and liquids other than blood in *Ae. aegypti*, and compared the results obtained by feeding from open drops with those obtained by feeding through membranes. They collected additional comparative data on liquids other than blood when offered through membranes to 494 mosquitoes. Their general conclusion was that hemoglobin in plasma or distilled water, whether taken from drops or through membranes, passed to the stomach and diverticula, with only the latter becoming fully distended. The authors noted also that "whereas *Aedes aegypti* will gorge freely on honey or glucose solutions from drops, either they are unaware of these fluids when presented in a membrane, or find difficulty in aspirating them."

Mixtures of blood with sweetened liquids have been fed to mosquitoes by Falleroni (1926), MacGregor and Lee (1929), MacGregor (1931), de Boissezon (1930), Roy and Ghosh (1940), Bishop and Gilchrist (1946), and Fisk (1950); two of these authors have furnished adequate experimental data.

Falleroni fed 40 *An. maculipennis* a mixture of citrated blood and sugar from open drops. On dissection liquid was found in the diverticula of all the mosquitoes; the stomachs were empty. Bishop and Gilchrist found that in all 37 *Ae. aegypti* fed on a blood-honey mixture from drops, the diverticula were fully distended, with only traces of liquid in the midgut. Five mosquitoes feeding on this same mixture through a membrane showed traces of liquid in the midgut only.

During routine dissections of *Anopheles* (*Anopheles*) *quadrimaculatus* Say fed in the course of human malaria investigations, it was noted in this laboratory that some of the individuals had blood in the diverticula as well as in the stomach, and that the distribution of the blood was not always the same. During previous dissections of *Aedes* (*Stegomyia*) *aegypti* (L), it had been noticed that the diverticula did not contain blood. In view of this, and because of the divergence of opinion among various authors, it was decided to obtain quantitative data on this point for *An. quadrimaculatus* and *Ae. aegypti*. Later, the experimental work was expanded to include limited data on *Anopheles* (*Anopheles*) *freeborni* Aitkin, *An.* (*Nyssorhynchus*) *albimanus* Wied., *An.* (*Anopheles*) *aztecus* Hoff., *Aedes* (*Stegomyia*) *albopictus* (Skuse), *Ae.* (*Finlaya*) *atropalpus* (Coq.), *Ae.* (*Aedimorphus*) *vexans* (Meig.), and *C.* (*Culex*) *pipiens* L.

MATERIALS AND METHODS

The mosquitoes used in these experiments were fed blood, glucose or honey solutions, mixtures of blood and glucose, and mixtures of blood and honey. Preliminary procedures were similar for all species of mosquitoes, i.e., those to receive blood meals were fed on fruit slices or glucose solutions prior to the experimental feedings and deprived of food and water for 18 to 24 hours before the test bites; those to be fed experimentally on glucose or honey solutions were deprived of food for about 48 hours and water for 24 hours. The experimental blood meals were the first taken by all of these mosquitoes except those used in the malaria investigations already referred to. Unless noted otherwise, insectary temperatures were maintained at 76°F. to 80°F., and relative humidity between 25 per cent and 50 per cent.

The mosquitoes obtained blood by biting man, by feeding through membranes, or from moistened squares of cellucotton. The glucose and honey solutions were colored with neutral red and offered through membranes or on cellucotton. Since there was no apparent difference in the destination of human blood and chicken blood, the mosquitoes were fed on heparinized chick blood whenever blood was offered through a membrane or from cellucotton. Except in those instances noted in the text, glucose was fed in 5 per cent solution. The blood-glucose and blood-honey mixtures consisted of 9 parts of heparinized whole blood and one part of 5 per cent glucose or honey in mammalian physiological saline (0.85 per cent).

For the membrane feedings, both Baudruche capping membranes (suggested by Dr. Dwight W. DeLong) and prepared chick skins were used. The latter were treated by soaking them in absolute alcohol for at least 30 minutes and then in distilled water for about one hour after the method of Bishop and Gilchrist (1946) just prior to their being used. Each membrane was fastened securely over one end of a glass cylinder, about 2.5 cm. in diameter and 6 cm. long. The cylinders were placed vertically on the bobbinet tops of small cages housing the mosquitoes. Test solutions, heated to approximately 45°C., were poured into the tubes and the mosquitoes allowed to feed.

After all experimental feedings the insects were chloroformed and dissected in physiological saline. They were routinely examined under a magnification of 70X, and in critical cases under oil immersion.

Significance was determined by chi square, which was calculated by means of the "fourfold" table, and the formula adjusted for small numbers.

RESULTS

Blood meals obtained by biting

Each *An. quadrimaculatus* female used in the experimental malaria investigations was allowed to insert its proboscis twice without feeding and then allowed to ingest a full blood meal on the third insertion. Approximately two and a half to three hours elapsed between feeding and dissection; the stomachs and diverticula were dissected out of 239 of these mosquitoes. The midguts of all 239 and the diverticula of 102 mosquitoes were partially or fully distended with blood.

Females of eight other species were allowed to bite without interruption until satiated, and were dissected immediately. The results are given in Table 1, A.

TABLE 1

Distribution of blood in the stomach and diverticula of mosquitoes dissected immediately after biting man (A) continuously until satiated, (B) with interruptions until satiated, and (C) continuously until partially fed

MOSQUITO SPECIES	NO. FED	LOCATION OF BLOOD		
		In stomach (No.)	In one or more sacs	
			No.	Per cent
A. *Continuous bite—satiation*				
An. quadrimaculatus	96	94[1] p, f	74 p, f	77
An. freeborni	90	90 f	15 s, p, f	17
An. albimanus	142	142 f	37 s, p, f	26
An. aztecus	87	87 f	32 s, p	37
Ae. aegypti	174	174 f	1 p	0.7
Ae. albopictus	40	40 p, f	0	0
Ae. atropalpus	41	41 f	8 s, p, f	19
Ae. vexans	20	20 p, f	0	0
C. pipiens	183	183 p, f	99 s, p, f	54
B. *Interrupted bite*[2]*—satiation*				
An. quadrimaculatus	21	21 f	15 p, f	71
An. freeborni	27	27 f	6 s, p	22
An. albimanus	50	50 p, f	16 s, p, f	32
Ae. aegypti	118	118 p, f	3 s, f	2
Ae. albopictus	26	26 p, f	0	0
C. pipiens	68	68 p, f	50 s, p	73
C. *Continuous bite—partial meal*				
An. quadrimaculatus	58	58 p	47 s, p	81
An. freeborni	27	27 p	7 s	26
An. albimanus	51	51 s, p	7 s, p	14
Ae. aegypti	102	102 s, p	7 s	7
Ae. albopictus	38	38 s, p	2 p	5
C. pipiens	70	70 p	31 s, p, f	44

s—slightly distended
p—partially distended
f—fully distended

[1] Two had empty stomachs but had blood in the diverticula

[2] Each female was disturbed at least twice before being allowed to finish biting

Of the 96 *An. quadrimaculatus*, 94 had blood in their stomachs and 74 of the 94 had blood in the diverticula. Of the two that had no blood in the stomach, one had a slight amount in the crop and one dorsal diverticulum, the other, a slight amount in the crop. Fifteen of the 90 fully-engorged *An. freeborni* had blood in

the diverticula, 10 had slightly or partially filled dorsal sacs, one a completely filled dorsal sac, and the other a completely filled ventral sac. Among the 142 *An. albimanus*, 37 had blood in the diverticula; in one of these, the crop was fully distended. Eighty-seven *An. aztecus* engorged, and 32 of these had blood in the diverticula. In only one of the 32 was there a full diverticulum; this one had a slightly distended dorsal sac and a fully distended crop.

Of 174 *Ae. aegypti*, one had a small amount of blood in one dorsal sac and in the crop. None of the *Ae. albopictus* had blood in the diverticula. The *Ae. atropalpus* was an autogenous species (Trembley, 1947) and consequently the females did not bite as readily as those requiring blood for reproduction; however, 41 individuals, in 2 series, were induced to bite, and they fed until their midguts were fully distended. Eight had blood in one or more diverticula; and in 2 of these all 3 sacs were partially filled, and in one, completely filled. Twenty *Ae. vexans* available for this group of tests fed; 19 had fully distended stomachs and the other one, partially distended. There was no blood in the diverticula.

Of the 183 *C. pipiens* biting until satiated, 8 were partially and 175 fully distended; 99 had blood in the diverticula. The midgut and all 3 sacs of one of these 99 were fully distended; the midguts were fully distended and all 3 sacs partially or slightly filled in 4 others.

The effect of interruption of feeding on the distribution of blood was studied in six species (Table 1, B and C). Two methods were followed: (1) mosquitoes were disturbed at least twice and then allowed to bite until satiated, and (2) mosquitoes when partially engorged were removed while in the act of biting. Dissections were made immediately. Blood was present in the diverticula of some satiated individuals of all species except *albopictus* (Table 1, B); it was present also in some partly fed mosquitoes of all the species studied (Table 1, C).

Observations were also made on the distribution of blood immediately, 2 hours, and 24 hours after the mosquitoes had fed. The insects were allowed to bite until satiated; then comparable lots were dissected at 0-, 2-, and 24-hour intervals. While biting and during the intervals, the mosquitoes were kept at 27°C. to 30°C. With the exception of *Aedes aegypti*, all species had blood in one or more diverticula at 0 hours and at 24 hours; although at the latter period the number of mosquitoes with blood present in these organs was definitely decreased (Table 2).

An unsuccessful attempt was made to induce 30 *An. quadrimaculatus* and 30 *Ae. aegypti* to bite when their abdomens were distended with eggs. Both species after partaking of one blood meal to activate the ovaries, refused to feed again until after oviposition had reduced the abdomen to normal size.

In order to determine without question what effect the absence of mature eggs might have on the distribution of blood, 18 female *An. quadrimaculatus* were reared in individual containers to prevent any possibility of fertilization, and then they were allowed only a partial meal to preclude any alleged "overflow" into the diverticula. On dissection all the stomachs were found to be partially filled. In 14 individuals (78 per cent) one or more diverticula contained blood.

Observations were made on 32 *An. quadrimaculatus*, 19 of which fed until engorged and then ejected a droplet of fluid, and 13 of which, although apparently just as fully distended, emitted no fluid. Of the 19 which ejected the

droplet, 15 (78 per cent) had blood in one or more diverticula; 3 of these had some blood in all 3 sacs. Of the 13 which did not eject, 8 (62 per cent) had one or more diverticula containing blood; only one had blood in all 3 sacs.

Blood meals imbibed from moistened cellucotton

A comparison was made of the distribution of heparinized blood when obtained without interruption from cellucotton by seven species (Table 3, A). The mosquitoes did not feed as readily from the cotton as from a living host.

TABLE 2

Distribution of blood in the stomach and diverticula of mosquitoes dissected at 0-, 2-, and 24-hour intervals after biting continuously to satiation

MOSQUITO SPECIES	DISSECTION INTERVAL (HRS.)	NO. FED	LOCATION OF BLOOD		
			In stomach	In one or more sacs	
				No.	Per cent
Anopheles quadrimaculatus	0	43	43 f	37 p	86
	2	20	20 f	14 p	70
	24	24	24 f	1 s	4
Anopheles freeborni	0	10	10 f	2 p, s	20
	2	10	10 f	2 p	20
	24	11	11 p, f	0	0
Anopheles albimanus	0	50	50 f	12 s, p	24
	2	50	50 (46 f)	16 s, p	32
	24	50	50 (10 f)	9 s, p	18
Aedes aegypti	0	25	25 f	0	0
	2	20	20 f	0	0
	24	20	20 f	0	0
Culex pipiens	0	26	26 (23 f)	19 s, p	73
	2	28	28 f	21 p	75
	24	28	28 (15 f)	10 s, p	36

s—slightly distended
p—partially distended
f—fully distended

Only 31 of 106 *An. quadrimaculatus*, 10 of more than 300 *An. freeborni*, and 35 of 46 *An. albimanus* were induced to feed in this manner. The diverticula in the *Anopheles* spp. were, on the whole, well-filled.

The *Ae. aegypti* imbibed freely, 59 of 78 feeding continuously to satiation and 40 of 57 feeding to satiation after 2 interruptions. Seven of the 59 undisturbed mosquitoes had blood in one or more diverticula; one of these 7 had a completely distended crop. Six of the 40 disturbed *Ae. aegypti* had traces of blood in one or more diverticula. The *Ae. albopictus* were offered blood on cellucotton many times, but only 20 fed out of the hundreds tried. Two of these 20 had blood in their diverticula; one had 3 sacs partially distended, the other, a slight amount in the crop. The *Ae. atropalpus*, also, were reluctant to imbibe. The same lot which fed avidly, when allowed to bite my arm, refused to approach the same blood when drawn from the arm and placed on cellucotton while still warm. Only 5

of more than 122 mosquitoes fed from cellucotton; and the stomachs and diverticula contained very small amounts of blood.

TABLE 3

Distribution of liquids in the stomach and diverticula of mosquitoes dissected immediately after obtaining (A) blood from moistened cellucotton, (B) glucose solutions from moistened cellucotton, and (C) glucose solutions through membranes. Feedings were continuous except in the group designated by *

MOSQUITO SPECIES	NO. FED / NO. EXPOSED	LOCATION OF LIQUID		
		In stomach (No.)	In one or more sacs	
			No.	Per cent
A. *Blood from moistened cellucotton*				
An. quadrimaculatus	31/106	20[1] (3 f)	27 (11 f)[2]	87
An. freeborni	10/+300	8 (2 f)	4 (2 f)	40
An. albimanus	35/46	35 (27 f)	11 (5 f)	31
Ae. aegypti	59/78	59 (58 f)	7 (1 f)	12
*Ae. aegypti**	40/57	40 (36 f)	6 s, p	15
Ae. albopictus	20/hundreds	20 (19 f)	2 s, p	10
Ae. atropalpus	5/+122	4 s	3 s	—
C. pipiens	67/91	66 (49 f)	38 (5 f)	57
B. *Glucose solutions from moistened cellucotton*				
An. quadrimaculatus	32/34	18 (1 f)	32 (24 f)	100
An. freeborni	20/21	1 p	20 (13 f)	100
An. albimanus	24/27	19 (1 f)	24 (13 f)	100
Ae. aegypti	35/65	18 (2 f)	33 (17 f)	94
Ae. albopictus	57/60	5 s, p	57 (40 f)	100
Ae. atropalpus	23/44	21 (8 f)	23 (18 f)	100
C. pipiens	39/44	24 (1 f)	39 (24 f)	100
C. *Glucose solutions through membranes*				
An. quadrimaculatus	27/100	19 (4 f)	26 (24 f)	96
An. freeborni	15/23	1 s	15 (14 f)	100
An. albimanus	22/+600	0	22 (15 f)	100
Ae. aegypti	44/59	40 (1 f)	38 (18 f)	86
Ae. atropalpus	4/40	4 s, p	2 (1 f)	—
C. pipiens	91/96	15 s, p	90 (38 f)	99

s—slightly distended
p—partially distended
f—fully distended
[1] Seven had empty stomachs but had blood in the diverticula
[2] Four had empty diverticula but had blood in the stomach
* Mosquitoes were interrupted before satiation

C. pipiens imbibed freely, 67 of 91 ingesting blood. Although one of these 67 had an empty stomach, it had a small amount of blood in all three diverticula.

Glucose solutions imbibed from moistened cellucotton

Considerable difficulty was experienced in getting sufficient numbers of females to feed immediately and continuously when offered glucose solutions on cellucotton; however, a large proportion of the mosquitoes were induced to feed in some lots of all 7 species. There was test fluid in one or more diverticula of every mosquito except 2 *Ae. aegypti* (Table 3, B); although these 2 had empty diverticula, each had a slight amount of the liquid in its stomach.

Glucose solutions obtained through membranes

Six species of mosquitoes were fed in this group of tests (Table 3, C). *Ae. albopictus* would not feed although hundreds of females were tried under varying conditions. The 5 per cent glucose solution, a 10 per cent glucose solution, and finally a syrupy liquid (made by boiling a super-saturated glucose solution) were offered and refused. *An. freeborni* and *An. albimanus* fed on the syrupy liquid only; *Ae. aegypti* took the 10 per cent solution readily; and the other two species fed on the 5 per cent solution.

In practically all instances, liquid was dispatched to the diverticula. There were only 5 mosquitoes with fully distended stomachs in the entire test, i.e., 4 *An. quadrimaculatus*, and one *Ae. aegypti*.

Mixtures of blood and sugary solutions obtained through membranes

For the purpose of determining whether the addition of sugary liquids to whole blood would cause the bulk of the mixture to enter the diverticula, *Ae. aegypti* females were allowed to feed through a membrane on blood-glucose and blood-honey solutions. Whole blood, heparinized, was used as a control. Two tests were carried out, and the results were similar. The midguts in nearly every instance were fully distended, whereas there was blood in only a small proportion of the diverticula (Table 4).

Miscellaneous observations

In 8 species of mosquitoes (*An. quadrimaculatus*, *An. freeborni*, *An. albimanus*, *An. aztecus*, *Ae. aegypti*, *Ae. albopictus*, *Ae. atropalpus*, and *C. pipiens*) random examinations under oil-immersion were made in order to determine whether entire red blood cells pass through the orifices into the diverticula, or whether these sacs contain plasma only. Erythrocytes were found intact in each of the diverticula in all species.

Peristalsis was observed to be general in the alimentary tract of all species except *C. pipiens*. In one mosquito of this species dissected 24 hours after biting, peristaltic action was faint but definite; the stomach was partially filled and one dorsal sac slightly filled with blood.

The possibility of a regular sequence in the filling of the diverticula was considered; the location of liquid in each species was listed for (1) crop alone, (2) dorsal sacs alone, and (3) a combination of crop and one or both dorsal sacs. The tabulation was made of the data on mosquitoes which had taken blood by bite, blood from cellucotton, glucose solutions from cellucotton, and glucose solutions through a membrane. When the blood was taken by bite, distribution

was random, i.e. equally or near-equally distributed among the diverticula in all species except *C. pipiens*. When the blood was imbibed from cellucotton the distribution was random in *An. freeborni* and *Ae. aegypti*, but not in *An. quadrimaculatus*, *An. albimanus*, and *C. pipiens*. Whether imbibed from cellucotton or

TABLE 4

Distribution of blood and mixtures of blood and sugary solutions in the stomach and diverticula of Aedes aegypti *dissected immediately after feeding through a membrane*

LIQUID	NO. FED / NO. EXPOSED	LOCATION OF LIQUID		
		In stomach (No.)	In one or more sacs	
			No.	Per cent
Blood	47/70	47 (45 f)	4 (2 f)	8
Blood / Glucose	55/70	55 f	10 (5 f)	18
Blood / Honey	44/70	44 (43 f)	11 (4 f)	25

s—slightly distended
p—partially distended
f—fully distended

TABLE 5

Distribution of blood and glucose solutions in the diverticula of mosquitoes of those species in which there appeared to be a definite distribution pattern

LIQUID AND MANNER OF FEEDING	SPECIES	IN CROP ALONE	IN DORSAL SACS ALONE	CROP PLUS ONE OR BOTH DORSAL SACS	TOTAL
Blood by bite	*C. pipiens*	30	16	139	185
Blood from cellucotton	*An. quadrimaculatus*	11	0	16	27
	An. albimanus	10	0	1	11
	C. pipiens	4	0	24	28
Glucose sol. from cellucotton	*An. freeborni*	7	13	0	20
	C. pipiens	0	0	39 (liquid in all 3 sacs)	39
Glucose sol. through a membrane	*An. freeborni*	12	0	3	15
	C. pipiens	6	0	84 (liquid in all 3 sacs of 72 mosquitoes)	90

taken through a membrane, the glucose solution was distributed at random in the diverticula of *An. quadrimaculatus* and *Ae. aegypti*, but not in *An. freeborni* and *C. pipiens*. Table 5 lists those species in which there appeared to be a pattern of distribution rather than a random distribution.

DISCUSSION

Blood, whether taken by bite or from cellucotton soaked in heparinized blood, was dispatched primarily, but not exclusively, to the stomach in *An. quadrimaculatus*, *An. freeborni*, *An. albimanus*, *An. aztecus*, *Ae. aegypti*, *Ae. albopictus* *Ae. atropalpus*, *Ae. vexans*, and *C. pipiens*. There was a difference in the distribution of the blood in the diverticula and stomach of the 9 species as follows:

1. In *An. quadrimaculatus*, *An. freeborni*, *An. albimanus*, *An. aztecus*, *Ae. atropalpus*, *C. pipiens* — to the stomach and to the diverticula;
2. *Ae. aegypti*, *Ae. albopictus*, *Ae. vexans* — with a few exceptions, to the stomach only.

TABLE 6

Species differences in the occurrence of blood in the diverticula of mosquitoes dissected immediately after biting continuously until satiated. The X^2 test was used to determine significance (+) and lack of significance (−)

SPECIES	*An. quadrimaculatus*	*An. freeborni*	*An. albimanus*	*An. aztecus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. atropalpus*	*C. pipiens*
An. quadrimaculatus	×	+	+	+	+	+	+	+
An. freeborni	+	×	−	+	+	−	−	+
An. albimanus	+	−	×	−	+	+	−	+
An. aztecus	+	+	−	×	+	+	−	+
Ae. aegypti	+	+	+	+	×	−	+	+
Ae. albopictus	+	±	+	+	−	×	±	+
Ae. atropalpus	+	−	−	−	+	−	×	+
C. pipiens	+	+	+	+	+	+	+	×

\+ denotes $P \leq .01$
± denotes $.01 < P \leq .02$

After the mosquitoes had obtained blood by uninterrupted biting, immediate dissection and subsequent tabulation showed that the percentage of mosquitoes having blood in their diverticula varied according to species (Table 1, A). This ranged from 77 per cent in *An. quadrimaculatus* to 0 per cent in *Ae. albopictus* and *Ae. vexans*. Because *Ae. vexans* was available in one small series only, too much importance was not attached to data on this species.

The differences in percentage were not in all cases related to taxonomic differences. *An. quadrimaculatus*, *An. freeborni*, and *An. aztecus*, all of the subgenus *Anopheles*, varied among themselves more than with *An.* (*Nyssorhynchus*) *albimanus*; but the closely allied *Ae.* (*Stegomyia*) *aegypti* and *Ae.* (*Stegomyia*) *albopictus* differed little in percentage, while *Ae.* (*Finlaya*) *atropalpus* differed with them more than with *An. freeborni*.

That the differences were species phenomena rather than generic was emphasized by the results of the X^2 tests (Table 6). This table shows that 8 differ-

ences lacked significance and that 18 were significant. An almost identical response was evidenced by the taxonomically unrelated *An. quadrimaculatus* and *C. pipiens*, both differing significantly from every other species. The taxonomically close *Ae. aegypti* and *Ae. albopictus*, with 2 exceptions, responded in a manner similar to other species listed in the table. The first of these exceptions involved the differences between these two species and *An. freeborni*, *Ae. aegypti* differing significantly, *Ae. albopictus* lacking significant difference; however, the probability (P) of the difference between *Ae. albopictus* and *An. freeborni* occurring by chance was approximately 15 in 1,000, very close to the upper limit of .01, or 1 in 100, for differences lacking significance. The other exception concerned *Ae. albopictus* and *Ae. atropalpus*; here again the difference was close to the limit for the group lacking significance, since P equalled .02, or 2 in 100.

The 8 pairs of species lacking significant differences are listed below, together with the values of P (obtained from X^2) and the percentage differences (calculated from Table 1, A). It should be noted that P and the percentage differences are statistically unrelated since significance and lack of significance were determined not from percentages but from the absolute observed and the expected numbers.

SPECIES COMPARED	P (FROM X^2 TESTS)	PERCENTAGE DIFFERENCES (FROM TABLE 1, A)
Ae. atropalpus vs. *Ae. albopictus*	.02	19
Ae. atropalpus vs. *An. aztecus*	.05	18
An. freeborni vs. *Ae. albopictus*	.015	17
An. albimanus vs. *An. aztecus*	.10	11
An. albimanus vs. *An. freeborni*	.10	9
An. albimanus vs. *Ae. atropalpus*	.50	7
An. freeborni vs. *Ae. atropalpus*	.60	2
Ae. aegypti vs. *Ae. albopictus*	.40	0.7

In the 18 pairs of species differing significantly (not listed), P values were less than .001 except in the difference between *An. aztecus* and *C. pipiens* where P was less than .01. Percentage differences ranged from +17 (*Ae. aegypti* vs. *An. freeborni*) to +77 (*Ae. aegypti* vs. *An. quadrimaculatus*). There was an overlap into this group of percentage differences by the percentage differences of the group lacking significance (listed above); the 3 higher differences, 17, 18, and 19 of this latter group extended into the lower range of the former. These aberrations might be ascribed to the comparatively small numbers of certain species used.

Further examination of the above data shows that while in 18 comparisons the differences are highly significant, the group of eight lacking significance merits further scrutiny. Here, the first three comparisons may be questioned and listed as "probably significant", because of the percentage overlap mentioned in the preceding paragraph and because P could be within the range of significance. The inclusion of *An. albimanus* vs. *An. aztecus* and *An. albimanus* vs. *An. freeborni*, is questionable also. Since *An. freeborni* is definitely significantly different

from *An. aztecus* ($P < .001$), it follows that *An. albimanus* should be different from either or both *An. aztecus* and *An. freeborni*. *An. albimanus* occupies an intermediate place between *An. freeborni* and *An. aztecus* (Table 1, A), and it is possible that the anomalous position of *An. albimanus* vs. *An. aztecus* and *An. albimanus* vs. *An. freeborni* may change with additional numbers and fit in with the pattern definitely established for *An. freeborni* vs. *An. aztecus*. The next comparisons, *An. albimanus* vs. *Ae. atropalpus* and *An. freeborni* vs. *Ae. atropalpus*, involve that species, *Ae. atropalpus*, in which the numbers are small, and so their inclusion in the group might be held in abeyance pending further experimentation. The remaining comparison, *Ae. aegypti* vs. *Ae. albopictus*, appears to be the only one lacking significance; additional study would undoubtedly strengthen their similarity. Each of the eight species discussed would perhaps fall into its own niche, and with one exception (*Ae. aegypti* vs. *Ae. albopictus*) would differ significantly from all others; but due to the individual variation within the species and among the species, this would occur only after hundreds, maybe thousands, of feedings and dissections.

Species difference was definite, although not so pronounced, when mosquitoes were fed from cellucotton soaked in heparinized blood (Table 3, A). The percentage of mosquitoes having blood in their diverticula ranged from 87 per cent in *An. quadrimaculatus* to 10 per cent in *Ae. albopictus*; this was similar to the range of percentages in mosquitoes taking blood by bite. The percentage differences were similar also, that between *Ae. albopictus* and *An. quadrimaculatus* being 77 per cent, and *Ae. albopictus* and *Ae. aegypti*, 2 per cent. No attempt was made to determine significance because of the lack of sufficient numbers.

The above results are hardly in agreement with Pawan (1937), whose experiments led him to state that, after *Ae. aegypti* had bitten, the sacs were always filled, whereas those of *An. tarsimaculatus* (= *aquasalis*) were empty. The results reported here agree, however, with those of Bishop and Gilchrist (1946), who found that the diverticula of *Ae. aegypti* were usually empty whether the blood was taken from drops or through a membrane, and with Lumsden (1947), who observed a similar response when *Ae. aegypti* obtained blood by bite.

When applied to experiments discussed in this paper, "interrupted feedings" refer to two different methods by which mosquitoes received blood meals: (1) mosquitoes allowed to bite, with at least two interruptions, until satiated, and (2) mosquitoes allowed to bite until blood was seen in the digestive tract, then interrupted and removed. It was not often possible to differentiate between the two methods when reviewing papers of other authors, therefore in my own experiments the two types of "interrupted" bites are treated separately (Table 1, B and C).

The number of mosquitoes with blood in the diverticula was significantly lower ($P < .01$) in the *C. pipiens* fed partial meals (1, C) than in those fed interrupted but complete meals (1, B). In all the other species the difference was not significant.

When interrupted biting until satiation was compared with continuous biting until satiation, slight increases in the numbers with blood in their diverticula were

noted (Table 1, A and B); these differences, however, were not significant. Interrupted biting did not cause diversion of blood to the diverticula in *Ae. aegypti*, as observed by Philip (1930) and MacGregor (1931).

When the blood was offered on cellucotton, most species were relucant to feed; therefore, *Ae. aegypti* was the only species in which interrupted and continuous feeding until satiation were compared (Table 3, A). The difference was not significant; this disagrees with the findings of MacGregor (1930) who believed that interruption during discontinuous feeding (imbibing from open drops or moistened cotton) caused blood to be dispatched to the diverticula.

Since there was blood in the diverticula of mosquitoes which had taken only a partial blood meal, the presence of blood in the diverticula could not be due to an over-distension of the midgut, as claimed by de Grandpré and Charmoy (1900), Lebredo (1904), and Roy (1927). Examination of Table 1, C reveals blood in the sacs of all species fed, from 81 per cent in *An. quadrimaculatus* to 5 per cent in *Ae. albopictus*.

Furthermore, except in *C. pipiens*, there seemed to be no regular sequence in the filling up of the diverticula when blood was obtained by bite, as observed by Roy (1927) working with *An. subpictus* and *An. stephensi*. His conclusions that the stomach must fill up first, then the dorsal sacs, and finally the crop, do not agree with my observations (Table 5). In all species, except *C. pipiens*, the blood was distributed at random; there were approximately as many mosquitoes which had some blood in the crop but none in the dorsal sacs, as there were with an empty crop and blood in one or both sacs. In *C. pipiens* there was a trend toward the dispatch of blood to the crop ahead of the dorsal diverticula; of the 185 mosquitoes with blood in one or more sacs, 16 had blood in their dorsal diverticula alone, 30 in the crop alone, and 139 in the crop and one or both dorsal sacs. The crop was not necessarily filled prior to the dispatch of blood to the dorsal sacs, a frequent occurrence being a slightly or partially filled crop combined with one or two slightly or partially filled dorsal sacs.

Blood taken from cellucotton was distributed at random in the diverticula of *An. freeborni* and *Ae. aegypti*. In *An. quadrimaculatus*, *An. albimanus*, and *C. pipiens*, there was blood in the crop alone of 41 per cent, 91 per cent, and 14 per cent, respectively; the remainder had blood in one or both dorsal sacs as well as in the crop.

That blood in the diverticula disappears with the passage of time has been agreed on by Schaudinn (1904), Eysell (1924), Patton and Cragg (1913), Genna (1921), Roy (1927), and Pawan (1937). It was found, under conditions prevailing in this laboratory, that approximately 24 hours were required for *An. quadrimaculatus* and *An. freeborni* to empty the diverticula (Table 2). This is somewhat at variance with the statements of some of the above authors. In a 24-hour period, *An. albimanus* and *C. pipiens* showed only a slight decrease, from 12 to 9, and from 19 to 10, respectively, indicative of a slow rate of clearance of the diverticula. Although *Ae. aegypti* were dissected at intervals in order to check on the remote possibility that the blood might pass from the stomach to the diverticula, no blood was found at 0, 2, or 24 hours.

Although Falleroni (1926) and James (1926) found that gravid *An. maculipennis* would bite when the abdomen was distended with eggs, the former observed that the blood was dispatched immediately to the stomach, whereas the latter stated that the blood went to the diverticula. (In view of subsequent taxonomic studies, it is possible that these two men were dealing with different members of the *maculipennis* complex.) Genna (1920, 1921) reported that *An. claviger* females with mature or nearly mature eggs could not bite until after oviposition. In my experiments neither *An. quadrimaculatus* nor *Ae. aegypti* after feeding once on blood, would bite again until oviposition had reduced the abdomen to normal size.

The absence of mature eggs did not ensure that blood would go entirely to the stomach. Eighteen unfertilized *An. quadrimaculatus* were allowed to bite until only partially full to avoid an "overflow" from a distended midgut, yet the diverticula of 14 of the 18 contained blood.

Intact red blood corpuscles, both human and chicken, were present in the diverticula of all species, whenever blood was found. This is in accord with the work of Fisk (1950), who observed incidentally that erythrocytes entered the crop in *Ae. aegypti*; it is not in accord, however, with de Boissezon (1930), who stated that erythrocytes enter the three diverticula with difficulty. His supporting observation of finding hemolyzed blood in a male which had fed on a mixture of blood and syrup does not prove the point, because hemolysis may have occurred prior to feeding, when the blood and syrup were mixed. It is of interest that Adler and Theodor (1926) dissected more than 4,000 sandflies; only infrequently was whole blood or even colored liquid found in the crop; the midgut contained blood.

During the course of our studies, peristalsis was observed in the alimentary tract of all species dissected, with the exception of *C. pipiens*; here it was noted in one specimen only. This phenomenon may or may not be correlated to the several instances in which this species has departed from the trends established by the other species. *C. pipiens* was the only species to differ significantly in the number of specimens with blood in their diverticula in the interrupted satiated group and interrupted partially fed group (Table 1, B and C); likewise it was the only species to have an indication of a sequence in filling of the diverticula; and it also showed less tendency to empty the diverticula in 24 hours (Table 2).

Sugary solutions taken through a membrane, or direct from cellucotton, went primarily, but not exclusively, to the diverticula in all species (Table 3, B and C). Approximately 44 per cent of the mosquitoes with solutions in the diverticula had them in the stomach also. This observation is not in accord with those of Nuttall and Shipley (1903) and of Genna (1920, 1921), who, referring to *Anopheles* spp. and *C. pipiens* and to *An. claviger*, respectively, stated that liquids other than blood pass through the diverticula enroute to the stomach. They, with many other authors, reported full diverticula and empty or nearly empty midguts after meals of sugary solutions. Our observations agree with those of Roy and Ghosh (1940), who, working with *An. subpictus*, *An. stephensi*, *Ae. aegypti*, and *C. fatigans* (= *quinquefasciatus*), recorded that 79 per cent of the mosquitoes imbibing

liquids other than blood from cotton had liquid in their stomachs, and with those of Bishop and Gilchrist (1946) who obtained similar results with *Ae. aegypti* feeding from open drops. The midguts, although in most cases well filled, were not as fully distended as the diverticula, or as full as when the mosquitoes had ingested blood. *An. freeborni*, *Ae. aegypti*, and *C. pipiens* fed readily on the sugar solutions through membranes. This observation on *Ae. aegypti* differs from that of Bishop and Gilchrist (1946).

Mixtures of whole blood and honey and of whole blood and glucose solutions were taken through a membrane by *Ae. aegypti* as readily as whole blood, and the mixture passed primarily to the stomach. There was a very slight increase in the number of mosquitoes with food in the diverticula when the sugary substances were added (Table 4). Again, this is not in accord with MacGregor (1930, 1931) and Bishop and Gilchrist (1946).

The experimental results reported in this paper are offered as indicative rather than conclusive. The lack of agreement with other workers might be due to differences in species, or strains within the species, or to differences in the conditions under which the experiments were performed.

Investigations along the lines described by Fisk (1950), who performed some feeding experiments while investigating the enzymatic digestion of blood, should be expanded and made more specific, since it is obvious that one cannot arbitrarily group these insects and refer to reactions or responses of "mosquitoes". This grouping, as well as insufficient numbers of mosquitoes observed, has been the outstanding weakness in the research reported by the majority of investigators.

Whether one or all of the diverticula aid in the digestion of blood and sugar solutions, or whether they function solely as storage pouches, is yet to be determined. Certainly, the dorsal sacs are comparatively small in size, offer little storage space, and have slight peristaltic action; the ventral diverticulum, however, is a much larger organ, and frequently exerts considerable force by its peristaltic movement.

Future work should include histological and physiological studies; and large-scale experiments should be performed on many species under comparable conditions. The age of the mosquitoes, the number of previous meals, and the time of day when fed, must all be considered, and no variations allowed in comparable tests. An aid to large-scale investigations would be the apparatus designed by Greenberg (1949) for simultaneously feeding several lots of mosquitoes through membranes.

SUMMARY

There is general agreement among investigators as to the way in which mosquitoes feed, but a divergence of opinion as to whether the distribution of a liquid in the alimentary canal depends on the manner of feeding, the kind of liquid ingested, or on a combination of both.

Experiments were performed on four species of *Anopheles*, four *Aedes*, and on *Culex pipiens*, to more thoroughly investigate this controversial subject.

Whole blood was fed by allowing the mosquitoes to bite, and to imbibe from saturated cellucotton; glucose solutions were fed from cellucotton and through membranes; and mixtures of whole blood and sugary solutions were fed through membranes.

Under all conditions, blood was dispatched primarily to the stomach, and with few exceptions, in smaller amounts to the diverticula. The proportion of mosquitoes showing blood in the diverticula appeared to be a distinguishing physiological characteristic of the species, and ranged from less than 11 per cent in *Aedes albopictus* to over 70 per cent in *Anopheles quadrimaculatus*.

Glucose solutions, regardless of species or manner of feeding, were found primarily but not exclusively in the diverticula. When mixtures of blood and honey were fed through membranes to *Aedes aegypti*, they went primarily to the stomachs, with traces found occasionally in the diverticula.

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REPORT OF A CASE OF HUMAN NASAL MYIASIS BY THE GREEN-BOTTLE FLY, *PHAENICIA SERICATA*, IN SAN BERNARDINO COUNTY, CALIFORNIA

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Phaenicia sericata (Meigen) has previously been reported as causing nasal myiasis in Tripolitania by Onorato (1922). According to Zumpt (1951) this fly is attracted to odorous sores. This species is one of several economically important sheep-maggots in the British Isles, South Africa and New Zealand. Man has been attacked by *P. sericata* in Europe, Africa and Asia resulting in a serious form of wound myiasis (James 1947).

CASE REPORT

On October 28, 1951, a well-developed, well-nourished, white female, 53 years of age, was hospitalized with the chief complaint of recurrent right upper quadrant pain. Subsequent physical and laboratory examinations resulted in the diagnoses of cholelithiasis and adenocarcinoma of the transverse colon. An exploratory laparotomy and a partial colostomy was performed at 8:00 a.m. on November 4, 1951 with the use of sodium pentothal, ethylene—oxygen and ether. At 2:30 p.m. on the same day, a sterile Levine tube with continuous suction was inserted through the left nasal opening. The tube was given the "routine examination" each hour. At 9:45 a.m. on November 6, 1951, the nurse discovered "minute white worms" crawling about the nasal opening. Upon discovery of the "worms" the tube was removed and warm physiological saline irrigations were administered and approximately fifty organisms were removed. A second Levine tube was inserted and there was no further recurrence of the "worms". The patient's eye, nose and throat history and physical examination were essentially negative upon entry, and there was no subsequent nasal infection reported during her stay in the hospital. The patient had a normal postoperative recovery and was discharged on November 18, 1951.

Six of the "worms" were sent to our laboratory for identification. The organisms were recognized to be early second instar fly larvae. Five of the larvae were reared to adults and the remaining larva (3rd instar) was preserved in ethyl alcohol. Rearing procedures were as follows: the maggots were placed in a clean jar and were supplied with gastrocnemius muscle that was dissected from a recently killed ground squirrel, *Citellus beecheyi*. The squirrel's leg was skinned and the muscle immediately removed, washed with tap water and placed in the rearing jar. The jar was covered with a fine grade of muslin and placed in an incubator at 80°F. No adult flies had access to the meat or the jar cover at any time. Prior to pupation, sterilized sand was added to the jar for the larvae to pupate in. Three larvae pupated November 14 and two a short time later. Four adult *P. sericata* emerged on November 23, 24, 25 and 30, respectively. The fifth adult did not emerge until after November 30.

The authors are indebted to Dr. Curtis Sabrosky of the U. S. National Museum for confirmation of identification. The preserved larva and three adults are now in the collections of the U. S. National Museum.

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BOOK REVIEWS

Global Epidemiology, a Geography of Disease and Sanitation, Volume 2, Africa and the Adjacent Islands. By JAMES STEVENS SIMMONS, TOM F. WHAYNE, GAYLORD W. ANDERSON AND HAROLD MACLACHLAN HORACK; associate author RUTH ALIDA THOMAS; and collaborators. 652 pp. with maps and illustrations. Philadelphia, J. B. Lippincott Company, 1951. Price: $15.00.

It is most appropriate that the second volume of this series of critical compilations on medico-sociologic intelligence dealing with the health and problems of Africa should appear at this time. Who knows but this continent may play a far more important rôle in the world's future than is generally realized or appreciated. Appropriately the volume is dedicated to Sir Patrick Manson who so wisely said: "Never refuse to see what you do not want to see, or what might go against your own cherished hypothesis, or against the views of authorities." In eight sections the importance of geography and climate, populations, environment and sanitation, health services and medical facilities, and diseases of the Nile Valley, the Ethiopian Highlands, East Africa, the Islands of the Indian Ocean, South Africa, Equatorial Africa, West Africa and Northern Africa are shown and detailed in a most readable form. One admires the skillful and accurate condensation of the enormous store of data and reports which had to be sifted, analyzed and appraised for accuracy and reliability. Suitable sketch maps illustrate the geographic distribution of certain important infections and explain their independence of political borders. The information presented is encyclopedic and consequently it is a reference volume not only for the expert epidemiologist, traveller and student of tropical medicine, but equally for the statesman and explorer for natural resources. The excellent quality of its content, its carefully prepared index and an appendix on health hints for the tropics make it a book which must be read, not merely placed on the library shelves of the specialists or teaching institutions. Since it covers the whole African continent and not merely the regions south of the Sahara, it strikes a better balance than the equally important documentation of the African problems presented in the well-known survey presented 14 years ago by Lord Hailey. Those who have always had a great interest in Africa must be deeply grateful to the authors for devoting the spare time of their busy lives to the arduous task of preparing such a splendid book.

K. F. MEYER

Approved Laboratory Technic, by JOHN A. KOLMER, EARLE H. SPAULDING, AND HOWARD W. ROBINSON and 18 collaborators. 5th edition, 1180 pp., 28 colored plates, 403 illustrations, cloth. New York, Appleton-Century-Crofts, Inc., 1951. Price $12.00.

As indicated in the preface, this compendium of laboratory technics is intended for and has been well received by "medical students, physicians, teachers, clinical pathologists and medical technicians" since the first (1931) edition. Over 150 pages of text, illustrations and color plates have been added since 1945. The many who have known this book will welcome this well revised edition.

This book, the "principles and technics" of medical laboratory methods, relegates clinical interpretation to a separate volume. The sections on syphilis serology, in which each of ten recognized methods is detailed completely, from antigen preparation to the reading of the test, are outstanding. The clinical pathologic methods (examination of the blood, urine, sputum, stomach contents, feces, and puncture fluids) and the sections on culture media and bacteriology have likewise been brought up to date. Deletion of details regarding bile examination and pneumococcus typing might have permitted addition of other newer material on the photometric erythrocyte count; Papanicolaou fixatives; comparative sensitivities of liver function tests; and rapid fermentation tests for bacteria. Mention of the availability of certain stable, standardized antigens, such as streptolysin-O for the antistreptolysin test and coccidioidin for skin tests would have been helpful.

The illustrations in the parasitology and mycology sections are well done, and both areas are more complete than those of most general texts. Less emphasis on wet mount iodine preparations for intestinal parasites is suggested, thus permitting additional detail and colored plates on thick smears for malaria.

The revised presentation of blood grouping is satisfactory. Some laboratory directors may find the suggestion that ". . . sera left over from clots of blood specimens submitted . . ." may be used for replenishing stocks of anti-A and anti-B blood grouping sera; or that "rinsing in distilled water is ideal but not practical," as suggested in the glassware section, is incompatible with accuracy in serology or chemistry. Little background material on Rh is presented with respect to classification and the CDE(cde) English terminology. Hence, the uninitiated would be at a loss to interpret Rh′ (Cde), or relate it to Rh_0 (cDe). The presentation of the Coombs and of anti-Rh tests is not indicative of the pitfalls often encountered by the inexperienced in the use of these methods.

The most disappointing section is that on photometry and its use in blood chemistry. It is not easy to present photometry in simple form. Nevertheless, the authors' assumption that "photometric . . . logarithmic scale readings plotted against concentration . . . will fall on a straight line" and that "Two or more analysts will always get the same results . . ." appear to be oversimplifications. No photometric method is given for phosphatase. Filter section for cholesterol does not follow the rules set forth in a preceding section. The only actual example of an optical density-concentration graph is given for the determination of protein by the biuret reaction (a straight line relationship). Yet, for the determination of creatinine by the Jaffé reaction (which does not follow Beer's law at any concentration of creatinine clinically encountered) no such graph is suggested. It is to be hoped that in the next printing the photometry-blood chemistry sections will undergo the extensive revision which makes this work an invaluable guide in so many fields.

NELL F. HOLLINGER

Tropical Nutrition and Dietetics, by LUCIUS NICHOLLS, M.D., Director, Division of Nutrition, Ceylon; Nutrition Adviser to Commissioner-General, South East Asia. 3rd edition: 476 pp., illustrated. London: Balliere, Tindall & Cox; Baltimore, The Williams & Wilkins Co., 1951. $6.00, 42/.

This latest edition of a book first published in 1938 "primarily for medical men who work among the masses in the tropics" contains clearly presented information which the author obviously knows from first-hand experience and in which he is vitally interested. It includes several valuable chapters regarding the identification and classification of tropical foodstuffs, the processing and preparation of food, food poisoning and the control of insect pests in cereals—information not ordinarily available in general books on nutrition. A thirteen-page food composition table includes many exotic tropical foods. The chapter on disorders of malnutrition emphasizes the multiple and interrelated nature of most deficiency syndromes.

In discussing dietary requirements he points out the danger of "urging the use of foodstuffs which are nutritionally desirable but economically impossible" and shows how "good growth and health may be obtained on far cheaper diets when most of the foodstuffs are of vegetable origin." Public health activities are very well discussed both in relation to dietary and nutritional surveys and the practical application of nutrition knowledge.

The content and presentation of the first eight chapters dealing with the basic principles of nutrition are less satisfactory. They contain biochemistry more elementary than necessary for "medical men," and many quotations from the current literature, especially those relating to animal experimentation, of uncertain application to man. The use of space for the pyridoxin, panthothenic acid, biotin and vitamin E contents of foodstuffs may be questioned. The statement that "thymine, folic acid and B_{12} produce a similar reticulocyte

response in patients with pernicious anemia" should mention that only B_{12} is effective in preventing the neurological manifestations of this condition. Considerable miscellaneous material of varying degrees of usefulness is included in the twenty-two appendices.

Serious factual and typographical errors have been kept to a minimum. The book will be useful not only for physicians but for all public health workers in tropical areas who are not familiar with the foodstuffs, cooking methods and specific nutrition problems of the tropics.

NEVIN S. SCRIMSHAW

The Zoology of Tapeworms, by ROBERT A. WARDLE AND JAMES ARCHIE MCLEOD, University of Manitoba, Winnipeg, Canada. 780 pp. with 419 illus. Minneapolis: Published for the University of Manitoba by the Minnesota Press, 1952. $12.50.

This volume describing the tapeworms of the world, with data brought together from some 3,000 references, is an outstanding contribution to the literature of parasitology. There are excellent sections on general morphology, life cycles, biology, origin and evolution, and history and classification of tapeworms. The taxonomic presentation is unusually well organized and illustrated, and keys for the identification of all known genera of the world and species of North America are included. The history of each group and what the author terms "recognition features" give one a most useful orientation. The illustrations in this book are excellent and they are especially designed to aid not only the taxonomist but the biologist interested in life cycles. Schematic diagrams are used to emphasize salient characteristics used in identification and classification.

This book fills the basic need that helminthologists and parasitologists have long recognized. Its scope and breadth make it of great value for the zoologist and ecologist. Teachers, research workers and graduate students will all find this book most useful.

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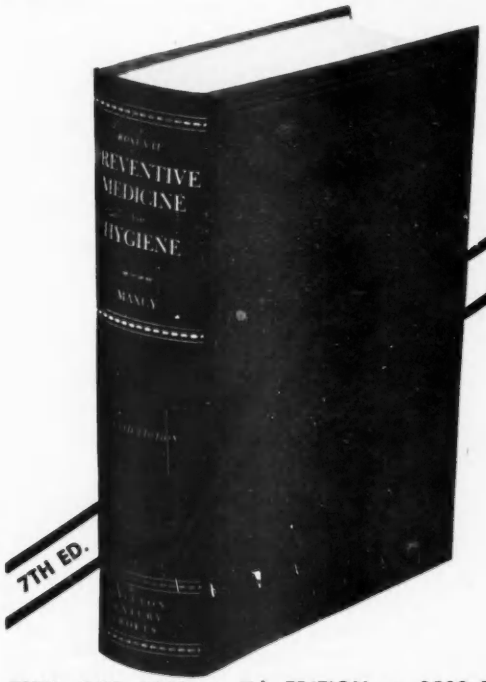

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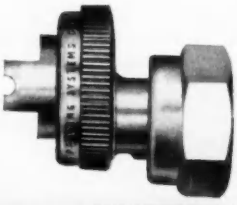

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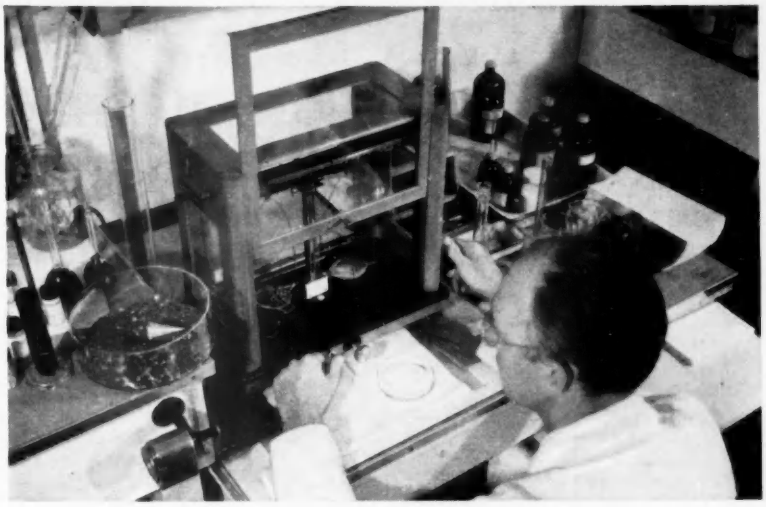

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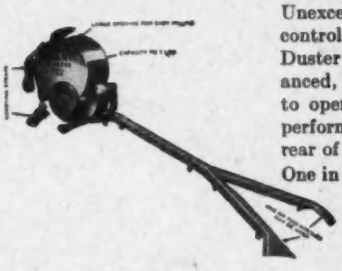

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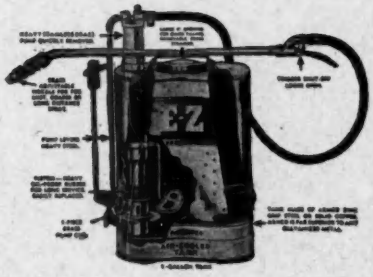

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